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(54) Title: NOGO AND NOGO RECEPTOR DERIVED PEPTIDES FOR T-CELL MEDIATED NEUROPROTECTION

(57) Abstract: An agent selected from: (a) a peptide derived from a Nogo or a Nogo receptor molecule and (b) autologous T cells recognizing a peptide of (a) in the context of class II MHC molecules, is provided for conferring neuroprotection and thus preventing or inhibiting neuronal degeneration in the central nervous system for ameliorating the effects of injury, disorder or disease.

NOGO AND NOGO RECEPTOR DERIVED PEPTIDES FOR T-CELL MEDIATED NEUROPROTECTION

FIELD OF THE INVENTION

The present invention is in the field of neurobiology and relates to peptides useful for eliciting a T-cell-mediated neuroprotective response.

ABBREVIATIONS: CFA: complete Freund's adjuvant; **CNS:** central nervous system; **EAE:** experimental autoimmune encephalomyelitis; **MBP:** myelin basic protein; **MHC:** major histocompatibility complex; **NgR:** Nogo receptor; **NS:** nervous system; **OVA:** ovalbumin; **PBS:** phosphate-buffered saline; **PNS:** peripheral nervous system; **RGC:** retinal ganglion cells; **SPD**: Sprague-Dawley; **TCR:** T-cell receptor.

BACKGROUND OF THE INVENTION

The majority of tissues in the body, including muscle, skin, liver and peripheral nerve, have the ability to repair themselves after injury. Strangely, however, the brain and spinal cord, which constitute the central nervous system (CNS), have little innate capacity for repair. In adults, most parts of the CNS cannot generate new neurons or regenerate new axons, the thin processes that relay electrical impulses between neurons. This lack of regenerative ability explains the devastating permanence of injuries to the CNS, as occurs in spinal-cord paralysis.

Substantial progress has been made in understanding why the injured CNS fails to regenerate and why the damage caused by the primary insult is propagated rapidly and irreversibly beyond the initial lesion.

In the search for answers to the inability of CNS nerve fibers to regrow, considerable attention has been directed to myelin-associated inhibitors. Based on

the observation that the myelin sheath around nerve axons inhibits neuronal regrowth, Schwab and colleagues identified two strongly inhibitory myelin proteins of molecular weight of about 35 kDa and 250 kDa, designated neurite growth inhibitors (NI-35 and NI-250, respectively) or neurite growth regulatory factors (Caroni and Schwab, 1988a; Schwab, 1996; WO 93/00427; US 5,684,133; US 6,103,232).

Schwab and colleagues then developed a monoclonal antibody named IN-1, which recognizes the proteins NI-35 and NI-250, and can neutralize the inhibition of axons by the oligodendrocytes and myelin in culture (Caroni and Schwab, 1988b; WO 93/00427). When they injected IN-1 into adult rats after spinal-cord injury, about 5% of severed axons regenerated across the injured tissue and the rats showed striking functional improvements (Bregman et al., 1995). For a decade, however, the identity of the molecules recognized by the IN-1 antibody has remained a mystery.

Then, recently, three groups (Chen et al., 2000; GrandPré et al., 2000; Prinjha et al., 2000) reported the identification in rats and humans of a hitherto unknown gene, *Nogo*, which encodes an inhibitory myelin protein. The breakthrough for all three groups came with the publication, by Schwab and colleagues (Spillmann et al., 1998), of six partial-peptide sequences of a bovine inhibitory molecule that is recognized by IN-1. Chen *et al.* then used a rat expressed sequence tag to probe for similar sequences in a rat complementary DNA (cDNA) library; Prinjha *et al.* performed a similar study to isolate the human cDNA; and GrandPré *et al.* found a full-length human cDNA that had previously been deposited in the Genbank sequence database. Based on homology, the protein encoded by all of these sequences, designated Nogo, turns out to be a fourth member of the reticulon family, which contains transmembrane proteins of unknown function.

There are three alternative isoforms of Nogo, designated Nogo-A, -B and –C.

Nogo-A is apparently the factor previously identified as the NI-250 inhibitor recognized by the IN-1 antibody (Bandtlow and Schwab, 2000; Brittis and Flanagan, 2001; Fournier and Strittmatter, 2001; Huber and Schwab, 2000; Qiu et al., 2000) and has the strongest inhibitory activity in culture. As expected, Nogo-A is localized

to the CNS myelin, and is highly expressed by oligodendrocytes but not by Schwann cells. Nogo-A was characterized as the antigen of the monoclonal antibody IN-1. When injected into the cerebrospinal fluid of rats after partial transection of their spinal cords, a recombinant humanized IN-1 fragment was reported to facilitate axonal regrowth and some functional recovery in rats after spinal cord injury (Brosamle et al., 2000).

Nogo-B and Nogo-C are found in certain neurons and several non-neural tissues (skeletal muscle, kidney, skin, lung, and spleen) and one of them may be the 35 kDa protein recognized by IN-1.

Rat Nogo-A has been fully characterized and its cDNA and gene have been cloned (Chen et al., 2000; WO 00/31235). The rat *nogo* gene was shown to encode at least three major protein products: the longer Nogo-A protein (1,163 amino acids) and two shorter Nogo proteins, Nogo-B (360 amino acids) and Nogo-C (199 amino acids) (Chen et al., 2000; WO 00/31235). The human isoforms of Nogo include the longer Nogo-A protein (1,192 amino acids; mol weight about 135 kDa) and two shorter Nogo proteins, Nogo-B (360 amino acids; ~ 37 K, lacks amino acids 186-1004 within the extracellular domain) and Nogo-C (199 amino acids; ~ 25 K, lacks amino acids 186-1004 but has a smaller, alternative N-terminal domain). Recombinant human Nogo-B, a stress-related protein associated with the endoplasmic reticulum, is described also in WO 00/60083. Recombinant human Nogo-C is described also in WO 01/36631.

Nogo-A has a 66-amino acid extracellular domain (Nogo-66) that interacts with a high-affinity receptor (NgR), a glycosylphosphatidylinositol (GPI)-linked protein with multiple leucine-rich repeats. Nogo-66 is expressed on the surface of oligodendrocytes and can inhibit axonal outgrowth trough the NgR. Nogo receptor proteins and peptides which block Nogo-mediated inhibition of axonal extension have been described (Fournier et al., 2001).

Studies of animal models of partial and complete CNS lesions performed by the present inventors have shown that after CNS injury, the white blood cells of the immune system (macrophages and T lymphocytes) can facilitate processes of

protection, repair, and regeneration (Hauben et al., 2000a; Lazarov Spiegler et al., 1996; Moalem et al., 1999; Rapalino et al., 1998).

Axonal injury to the spinal cord evidently activates an anti-self (autoimmune) response mediated by T cells directed against myelin-associated antigens. This response was found to be beneficial for the injured nerve, as it can reduce the post-traumatic degeneration, an otherwise inevitable consequence of the injury (Yoles et al., 2001). The ability to exhibit a protective autoimmune T cell response is genetically controlled, and is inversely related to the inherent resistance of the individual or strain to the development of an autoimmune disease, such as experimental autoimmune encephalomyelitis (EAE), when challenged with myelin-associated antigens (Kipnis et al., 2001). Moreover, the neuroprotective T cell response is amenable to boosting by passive or active immunization. Passive transfer of T cells reactive to myelin basic protein (MBP) significantly improves recovery from spinal cord contusion in rats (Hauben et al., 2000a, 2000b).

In seeking a way to convert the experimental immunization into an effective post-traumatic therapy, we have been testing peptides that are "safe" (i.e., do not induce autoimmune disease) and are derived from or cross-react with self-proteins (Hauben et al., 2001; Kipnis et al., 2000; Schori et al., 2001). We found that vaccination with MBP or a spinal cord homogenate containing a variety of myelin proteins promotes morphological and functional recovery of the injured spinal cords of both EAE-resistant and EAE-susceptible rats (Hauben et al., 2001). This finding raised another question: would vaccination with myelin antigens that inhibit axonal outgrowth be similarly (or more) effective or is the effect unique to a specific type of myelin protein?

In the laboratory of the present inventors, it has recently been discovered that activated T cells that recognize an antigen of the nervous system of the patient confer neuroprotection. Reference is made to US Applications Serial Nos. 09/218,277 and 09/314,161 and PCT Publication WO 99/60021, the entire contents of which is hereby incorporated herein by reference. More specifically, T cells reactive to MBP were shown to be neuroprotective in rat models of partially crushed optic nerve (see

also Moalem et al, 1999) and of spinal cord injury (see also Hauben et al, 2000a). Until recently, it had been thought that immune cells do not participate in NS repair. Furthermore, any immune activity in the context of CNS damage was traditionally considered detrimental for recovery. It was quite surprising to discover that nervous system-specific activated T cells could be used to protect nervous system tissue from secondary degeneration which may follow damage caused by injury or disease of the CNS or peripheral nervous system (PNS). The mechanism of action of such nervous system-specific T cells has yet to be discovered, but the massive accumulation of exogenously administered T cells at the site of CNS injury suggests that the presence of T cells at the site of injury plays a prominent role in neuroprotection.

In addition to the nervous system-specific activated T cells, the above-referenced US applications and PCT publication WO 99/60021 disclose that therapy for amelioration of effects of injury or disease of the CNS or PNS can be carried out also with a natural or synthetic CNS/PNS-specific antigen such as MAG, S-100, β-amyloid, Thy-1, P0, P2, a neurotransmitter receptor, and preferably human MBP, human proteolipid protein (PLP), and human oligodendrocyte glycoprotein (MOG), or with a peptide derived from said antigen. PCT/IL02/00032 describes modified peptides of a CNS- or PNS-specific antigen for use in neuroprotection therapy.

The myelin-associated protein Nogo-A has received more research attention than any other inhibitor of axonal regeneration in the injured CNS. Circumvention of its inhibitory effect, using antibodies specific to Nogo-A, particularly the monoclonal antibody IN-1, has been shown to promote axonal regrowth.

SUMMARY OF THE INVENTION

The present invention relates, in one aspect, to the use of an agent selected from: (a) a peptide derived from a Nogo or a Nogo receptor molecule and (b) autologous T cells recognizing a peptide of (a) in the context of class II MHC molecules, for the manufacture of a pharmaceutical composition which elicits a T-cell-mediated neuroprotective response.

The peptide may be derived from a mammalian Nogo or Nogo receptor protein such as, but not limited to, rat, bovine or human Nogo-A, Nogo-B or Nogo-C and mouse or human Nogo receptor.

In one preferred embodiment, the peptide is derived from the Nogo-A molecule such as the peptide herein designated p472, a 18-mer peptide consisting of the amino acid residues 623-640 of the rat Nogo-A and having the sequence of SEQ ID NO:2, or a modification of said peptide.

In another aspect, the present invention relates to a pharmaceutical composition which elicits a T-cell-mediated neuroprotective response comprising a pharmaceutically acceptable carrier and an agent selected from: (a) a peptide derived from a Nogo or a Nogo receptor molecule and (b) autologous T cells recognizing a peptide of (a) in the context of class II MHC molecules.

The pharmaceutical composition will preferably comprise an adjuvant that induces a Th-1 response to the peptide.

In a further aspect, the present invention provides a method for conferring neuroprotection and thus preventing or inhibiting neuronal degeneration in the central nervous system (CNS) for ameliorating the effects of injury, disorder or disease, comprising administering to an individual in need at least one agent selected from the group consisting of: (a) a peptide derived from a Nogo or a Nogo receptor molecule and (b) autologous T cells recognizing a peptide of (a) in the context of class II MHC molecules.

The peptides and T cells of the invention are indicated for neuroprotection in the CNS or for protecting CNS cells from glutamate toxicity in an area of myelinated axons, and thus for the treatment of an injury, disorder or disease of the CNS in order to prevent or inhibit neuronal degeneration or for treating an injury, disorder or disease in an area of myelinated axons of the CNS caused or exacerbated by glutamate toxicity.

A CNS injury, disorder or disease may be a damage to the CNS as result from a traumatic injury, such as penetrating trauma or blunt trauma, spinal cord injury, or a disease or disorder, including but not limited to Alzheimer's disease, Parkinson's

disease, multiple sclerosis, Huntington's disease, amyotrophic lateral sclerosis (ALS), glaucoma, senile dementia, stroke and ischemia.

BRIEF DESCRIPTION OF THE FIGURES

Fig. 1 is a graph showing that active post-traumatic vaccination with the Nogo-derived peptide p472 reduces paralysis after incomplete spinal cord injury in SPD rats. Female SPD rats were subjected to spinal contusion at T8 inflicted by the NYU impactor with a 10-g weight dropped from a height of 50 mm. Immediately after contusion, rats in one group (n = 6) were vaccinated with p472 emulsified in CFA and rats in another group (n = 6) were injected with PBS only. Motor behavior was assessed weekly in an open field by observers blinded to the treatment received by the rat. Recovery was best in rats vaccinated with p472. Results are mean values of the motor score ± SEM. Significance of the difference between the groups was determined using a two-tailed Student's *t* test (*$p < 0.05$; **$p < 0.01$).

Figs. 2A-2C are graphs showing a comparison between vaccination with the Nogo-derived peptide p472 and the MBP-derived altered peptide A91 in male SPD rats. (2A) Male SPD rats were subjected to spinal contusion as described for Fig. 1. Immediately after the contusion the animals were randomly divided into two groups (n = 6 in each group). Rats in the first group were immunized with p472 in CFA and rats in the second group were injected with PBS in CFA. Motor behavior was assessed as described for Fig. 1. Results are mean values of the motor score ± SEM. (2B) Male SPD rats were injured and grouped as in 2A, and were immunized with A91 in CFA or injected with PBS in CFA. Motor recovery in the A91-treated group, assessed as in 2A, was at least as good as with p472 treatment. Results are mean values of the motor score ± SEM. (2C) Assessment of recovery from a milder spinal contusion inflicted by dropping the 10-g weight from a height of 25 mm (*$p < 0.05$; **$p < 0.01$).

Figs. 3A-3B are photographs showing retrograde labeling of cell bodies in the red nucleus. Three months after spinal contusion, rats from each group were re-anesthetized and the dye rhodamine dextran amine (Fluoro-ruby) was applied below

the site of contusion. Five days later the rats were killed and their brains were excised, processed, and cryosectioned. Sections taken through the red nucleus were inspected and analyzed by fluorescence confocal microscopy. The photographs are of p472-immunized rats and of the control rats shown in Fig 2C.

Figs. 4A-4B show presence of proliferating T cells specific to p472 and absence of anti-p472 antibodies 1 week after vaccination with p472. (4A) The proliferative response of splenocytes prepared from rats injected with p472 in CFA or with PBS in CFA to their specific antigen was compared to their proliferative response to the non-specific antigen (OVA) in CFA or in the absence of antigen. Results are expressed as the mean cpm values of quadruplicate samples obtained from three different animals ± SEM. (4B) Antibodies in the treated rats were assayed by ELISA. The amount of antibodies detected in the rats immunized with p472 in CFA was not significantly greater than in those injected with PBS in CFA. Results are expressed by mean values (expressed in arbitrary units) ± SEM obtained from three rats, each tested in triplicate at each of the indicated dilutions.

Fig. 5 is a graph showing that passive transfer of p472-specific T cells into spinally contused rats promotes recovery. Male SPD rats were subjected to spinal contusion as described for Fig. 1. Immediately after contusion the rats were divided into two groups (n = 5 in each group). Rats in one group were injected intraperitoneally with p472-specific T cells (1×10^7 cells) in CFA, and rats in other groups were injected with PBS only. Motor behavior was assessed in an open field by observers blinded to the treatment received. Results are mean values of the motor score ± SEM. Significance of the differences between the groups at each time point was determined using a two-tailed Student's *t* test (*$p < 0.05$; **$p < 0.01$).

Figs. 6A-6B show that immunization with p472 reduces retinal ganglion cell (RGC) loss following partial crush injury of the rat optic nerve. (6A) Rats were subjected to a calibrated crush injury of the optic nerve immediately before or 5 days after injection with p472 in CFA, PBS in CFA, or T cells directed to p472. (6B) Rats were immunized with A91 in CFA or PBS in CFA immediately after optic nerve

crush. In both (A) and (B), the optic nerves were re-exposed 2 weeks later and a dye was injected distally to the primary lesion. After 5 days, the retinas were excised and whole-mounted, and the retinal ganglion cells were counted by observers blinded to the treatment received by the rats. Results are expressed as the mean numbers of RGCs per $mm^2 \pm$ SEM.

DETAILED DESCRIPTION OF THE INVENTION

The outcome of spinal cord injury is far more severe than one might predict based on the immediate effect of the insult because the injury not only involves primary degeneration of the impacted neurons, but also spreads by a self-destructive process that leads to secondary degeneration of surrounding neurons that escaped the initial insult. Interestingly, however, concomitant with the onset of secondary degeneration, a spontaneous signal is transmitted systemically to the immune system where it evokes an adaptive immune response associated with nerve protection and maintenance.

This response is very similar to that evoked by pathogen attack, against which recruitment of the immune system is considered essential. In the context of non-pathogenic damage in the CNS, recruitment of the adaptive immune system has not been considered an issue, since there seems to be no obvious need to mount a defense. Surprisingly, however, the present inventors found that even with non-pathogenic damage, such as that occurring in CNS trauma, an anti-self immune response is evoked, with the purpose of halting the progression of damage. Passive and active immunization with self-antigens normally found in the body can have a therapeutic effect by boosting any endogenous immune response to damage.

Thus, the present invention provides pharmaceutical compositions comprising an antigen being a synthetic CNS peptide derived from a Nogo or a Nogo receptor molecule that elicits a T-cell-mediated neuroprotective response and thus can be used for reducing or inhibiting the effects of injury or disease that result in CNS degeneration.

According to the present invention, an agent selected from: (a) a peptide derived from a Nogo or a Nogo receptor molecule and (b) autologous T cells recognizing a peptide of (a) in the context of class II MHC molecules, is provided for the manufacture of a pharmaceutical composition that elicits a T-cell-mediated neuroprotective response.

Nogo proteins, previously termed neurite growth regulatory factors, are CNS myelin-associated proteins which inhibit neurite outgrowth. Nogo and fragments thereof have been shown to inhibit neurite outgrowth in nerve cells and neuroblastoma cells and to inhibit the spreading of fibroblasts and melanoma cells, and have been proposed for treatment of melanoma, neoplastic diseases of the CNS, e.g. neuroblastoma, or tumors metastatic to the brain, e.g., glioblastoma. Antagonists of the Nogo proteins, particularly the antibodies IN-1 or IN-2, neutralize the neurite growth inhibitory factors and were proposed for use in the diagnosis and therapy of nerve damage resulting from trauma, infarction and degenerative disorders of the CNS (see WO 00/31235; US 5,684,133; US 6,103,232). A recombinant humanized fragment of the monoclonal antibody IN-1 was reported to facilitate axonal regrowth and some functional recovery in rats after spinal cord damage (Brosamle et al., 2000).

Nogo-A is a protein molecule that spans the membrane of the oligodendrocyte, one of the glial cell types in the brain. A 66-amino acid segment (called Nogo-66), expressed on the outside of the glial cell, causes a receptor-mediated inhibition of nerve growth. By using the Nogo-66 fragment, the Nogo receptor (NgR) was cloned and characterized. NgR was shown not only to bind Nogo-66 but also to cause its inhibitory effect (Fournier et al., 2001). This finding lead to the search of factors that would disrupt the interaction of Nogo with its receptor in order to facilitate axonal regeneration in vivo.

The present invention is based on a completely different effect, namely to vaccinate with a peptide derived from Nogo or NgR in order to elicit a T-cell-mediated neuroprotective response or to use passive vaccination with anti-Nogo T cells that recognize the peptide in the context of class II MHC molecules. This

neuroprotection of the CNS will prevent or inhibit neuronal degeneration caused by an injury, disorder or disease as well as to treat an injury, disorder or disease caused or exacerbated by glutamate toxicity.

Previous findings of the present inventors have demonstrated that active or passive immunization of CNS-injured rats or mice with myelin-associated peptides such as MBP induced a T-cell-mediated protective autoimmune response, which promotes recovery by reducing post-traumatic degeneration.

Although Nogo is a myelin antigen, the findings of the present invention are unexpected because Nogo and its receptor, contrary to MBP, are inhibitors of axonal outgrowth.

The peptide for use in the present invention is derived from a mammalian Nogo molecule such as, but not limited to, bovine Nogo (Chen et al., 2000) or rat Nogo-A (SEQ ID NO:1) (1,163 amino acids; database accession number AJ242961), rat Nogo-B (SEQ ID NO:3) (360 amino acids; AJ242962), rat Nogo-C (SEQ ID NO:4) (199 amino acids; AJ242963), human Nogo-A (SEQ ID NO:5), human Nogo-B (SEQ ID NO:6), and human Nogo-C (SEQ ID NO:7), or from a mammalian Nogo receptor (NgR) such as human or mouse NgR (Fournier et al, 2001) (SEQ ID NOs:9 and 10, respectively).

In one preferred embodiment, the Nogo molecule is rat Nogo-A (SEQ ID NO:1) or human Nogo-A (SEQ ID NO:5) and the peptide is the peptide designated p472 (SEQ ID NO:2) containing the residues 623-640 of rat Nogo-A, of the sequence identified herein as SEQ ID NO:2 :

S Y D S I K L E P E N P P P Y E E A (SEQ ID NO:2)

In another embodiment, the peptide is derived from the Nogo receptor and is selected from the sequences identified herein as SEQ ID NOs:10-11:

S G V P S N L P Q R L A G R D (SEQ ID NO:10)

T R S H C R L G Q A G S G S S (SEQ ID NO:11)

Also encompassed by the present invention are peptides of the SEQ ID NO:2, SEQ ID NO:10 and SEQ ID NO:10 modified by deletion, addition or replacement of one or more amino acids.

As used herein, the term "peptide derived from a Nogo or a Nogo receptor molecule" refers both to (i) a peptide having a sequence comprised within the sequence of a Nogo or a Nogo receptor molecule; and (ii) a peptide of (i) modified by replacement of one or more amino acid residues by different amino acid residues at the T cell receptor (TCR) binding site and not at the MHC binding site(s), so that the immune response is activated but not anergized.

The peptides of the invention have at least 9-50, preferably 10-30, more preferably 10-20, most preferably 13-18 amino acid residues. The modified or altered peptides may be produced by replacement of one or more amino acid residues of the peptide by other amino acid residues, preferably in their TCR binding site. Suitable replacements are those in which charged amino residues like lysine, proline or arginine are replaced by glycine or alanine residues. For example, altered peptides can be produced from the above p472 Nogo peptide (Nogo p623-640) of SEQ ID NO:2 herein by replacement of the Lys 628 residue and from the Nogo receptor of SEQ ID NOs:10-11 herein by replacement of the Arg (R) residue by Val or Ala or another similar residue.

In order to obtain the desired T-cell-mediated neuroprotective response, the peptide of the invention is administered in a composition containing at least one adjuvant that induces a Th-1 response to the peptide.

Any adjuvant that induces a Th-1 response to the peptide is encompassed by the invention including, but not being limited to, at least one adjuvant selected from lipopolysaccharide (LPS), a synthetic oligodeoxynucleotide containing CpG motifs, a killed bacterium such as Brucella abortus, Mycobacterium vaccae or BCG, a bacterial toxin such as tetanus toxoid, Pseudomonas aeruginosa toxin A or Bordetella pertussis toxin, beta-galactosidase, influenza virus hemagglutinin and nucleoprotein, hepatitis B core and surface antigens, block copolymer adjuvant P1005, Algammulin and gamma-inulin.

In order to obtain the Th-1 response, the peptide may also be covalently associated with a lipopeptidic immunostimulant, encapsidated into liposomes or is admixed with controlled-release microparticles or with a immune-stimulating complex (iscom) matrix.

In another embodiment of the invention, passive vaccination is carried out with autologous T cells which recognize the peptide derived from a Nogo molecule or a Nogo receptor in the context of class II MHC molecules.

Circulating T cells of a subject which recognize the peptide, usually after active immunization with said peptide, are isolated and expanded using known procedures. For administration, the T cells are isolated, preferably are activated by the peptide and are then expanded by a known procedure. The T cells can be used for treatment after culture or may be stored by standard procedures for later use.

The pharmaceutical compositions for use in accordance with the present invention may be formulated in a conventional manner using one or more physiologically acceptable carriers or excipients. The carrier(s) must be "acceptable" in the sense of being compatible with the other ingredients of the composition and not deleterious to the recipient thereof.

Methods of administration include, but are not limited to, parenteral, e.g., intravenous, intraperitoneal, intramuscular, subcutaneous, mucosal (e.g., oral, intranasal, buccal, vaginal, rectal, intraocular), intrathecal, topical and intradermal routes. Administration can be systemic or local.

According to the present invention, we examined whether post-traumatic immunization with a peptide derived from the myelin-associated growth-inhibitory protein Nogo-A (p472) can promote recovery from spinal cord injury. If so, we were interested in finding out whether such peptides can reduce the spread of damage without risk of inducing an autoimmune disease, and whether the effect is mediated by antibodies or by T cells.

It is shown herein in the application that neuronal degeneration after incomplete spinal cord contusion in rats was substantially reduced, and hence recovery was significantly promoted, by post-traumatic immunization with the

Nogo-A derived p472 peptide. The observed effect appeared to be mediated by T cells and could be reproduced by passive transfer of a T cell line directed against the Nogo-A peptide. It thus appears that after incomplete spinal cord injury, immunization with a peptide derived from Nogo-A, can be used to evoke a T cell-mediated response that promotes recovery.

The choice of peptide(s) for clinical treatment of spinal cord injuries should be based on safety considerations, in particular the likelihood that the chosen peptide will not cause an autoimmune disease or interfere with essential functions of this peptide or other proteins. From a therapeutic point of view, the fact that the active cellular agents are T cells rather than antibodies is an advantage, as T cell production commences within the time window required for a protective effect after spinal cord injury, whereas antibody production takes longer.

T cell-mediated active or passive vaccination is a promising treatment for post-traumatic treatment of the injured spinal cord, since it is based on harnessing of the immune system. Because the active players are immune cells and not antibodies, this type of therapy is likely to provide multifactorial protection.

As used herein, the term "neuroprotection" refers to prevention or inhibition of degenerative effects in the CNS caused by an injury, disorder or disease or caused or exacerbated by glutamate toxicity.

The peptides and T cells of the invention may be used for neuroprotection in the CNS or for protecting CNS cells from glutamate toxicity, particularly in an area of myelinated axons. In this context, they are useful for treating an injury, disorder or disease of the CNS in order to prevent or inhibit neuronal degeneration or for treating an injury, disorder or disease in an area of myelinated axons of the CNS caused or exacerbated by glutamate toxicity.

In one embodiment, the injury, disorder or disease may be, without being limited to, spinal cord injury or brain injury, blunt trauma, penetrating trauma, brain coup or contrecoup, hemorrhagic stroke, or ischemic stroke. In one preferred embodiment, the injury to be treated is spinal cord injury.

In another embodiment, the injury, disorder or disease may be, without being limited to, a senile dementia including Alzheimer's disease, Lewy Body disease, and multi-infarct dementia, Parkinsonian syndrome including Parkinson's disease, facial nerve (Bell's) palsy, Huntington's disease, a motor neuron disease including amyotrophic lateral sclerosis, a prion disease including Creutzfeldt-Jakob disease, Alper's disease, Batten disease, Cockayne syndrome, status epilepticus, epilepsy, multiple sclerosis, amnesia, seizures, oxidative stress, opiate tolerance and dependence, and glaucoma. In one preferred embodiment, the disease to be treated is glaucoma.

The invention will now be illustrated by the following non-limiting examples.

EXAMPLES

Materials and Methods

Animals. Inbred adult Sprague-Dawley (SPD) and Lewis rats (10–12 weeks old, 200–250 g) were supplied by the Animal Breeding Center of The Weizmann Institute of Science (Rehovot, Israel). The rats were matched for age and weight in each experiment and housed in a light- and temperature-controlled room.

Antigens. Myelin basic protein (MBP) and ovalbumin (OVA) were purchased from Sigma (St. Louis, MO, USA). Altered (non-encephalitogenic) MBP peptide (A91) was derived from an encephalitogenic peptide, amino acids 87–99 of MBP, by replacing the lysine residue 91 with alanine (VHFFANIVTPRTP) (Gaur et al., 1997). Both A91 and the Nogo-A-derived peptide p472 (SYDSIKLEPENPPPYEEA) (Chen et al., 2000) were synthesized at the Weizmann Institute of Science (Rehovot, Israel). Antigens were emulsified in equal volumes of complete Freund's adjuvant (CFA; Difco Laboratories, Detroit, MI, USA) supplemented with *Mycobacterium tuberculosis* (1 mg/ml, Difco).

Spinal cord contusion or transection. Rats were anesthetized by intraperitoneal injection of Rompun (xylazine, 10 mg/kg; Vitamed, Israel) and Vetalar (ketamine,

50 mg/kg; Fort Dodge Laboratories, Fort Dodge, IA, USA) and their spinal cords were exposed by laminectomy at the level of T9. One hour after induction of anesthesia, a 10-g rod was dropped onto the laminectomized cord from a height of 25 mm or 50 mm, using the NYU impactor, a device shown to inflict a well-calibrated contusive injury of the spinal cord (Young, 1996). The spinal cords of another group of rats were completely transected, as previously described (Rapalino et al., 1998).

Active immunization. Rats were immunized subcutaneously in the base of the tail, on a random basis, with 150 μg of p472 or A91, or were injected with phosphate-buffered saline (PBS), each emulsified in an equal volume of CFA containing 1 mg/ml bacteria. Seven days later, each rat was injected subcutaneously with a booster of 100 μg of the same antigen emulsified in incomplete Freund's adjuvant (IFA; Difco). The first immunization was given not later than 1 h after contusion. Control rats were sham-operated (laminectomized but not contused), immunized, and examined daily for symptoms of EAE, which were scored on a scale of 1 to 5 (Ben-Nun and Cohen, 1982).

SPD T cell line specific to p472. T cell line was generated from draining lymph node cells obtained from immunized rats as described (Gillis et al., 1978). Cells were grown in propagation medium for 4–10 days before being restimulated with their antigen (10 μg/ml) in the presence of irradiated (2000 rad) thymus cells (1×10^7 cells/ml) in proliferation medium. The T cell line was expanded by repeated stimulation and propagation.

Animal care. In contused rats, bladder expression was assisted by massage at least twice a day (and 3 times a day during the first 48 h after injury), until the end of the second week, by which time SPD rats had recovered automatic voidance. Lewis rats required this treatment throughout the experiment. All rats were carefully monitored

for evidence of urinary tract infection or other signs of systemic disease. During the first week after contusion and in any case of hematuria thereafter, they received a course of sulfamethoxazole (400 mg/ml) and trimethoprim (8 mg/ml) (Resprim, Teva, Petah Tikva, Israel), administered orally by syringe (0.3 ml of solution per day). Daily inspections included examination of the laminectomy site for evidence of infection and assessment of the hind limbs for signs of autophagia or pressure.

Proliferation assay. Female SPD rats were immunized with p472. Ten days later lymph nodes were excised, dissociated, and the washed lymphocytes (2×10^6 cells/ml) were cultured in 0.2 ml of proliferation medium (Hauben et al., 2000a). The cells were activated for 72 h at 37 C°, 90% relative humidity and 7% CO_2 in the in the presence of irradiated thymocytes (2000 rad, 2×10^6 cells/ml), together with p472 (10 μg/ml), A91 in CFA (10 μg/ml), the non-self antigen ovalbumin (10 μg/ml), concanavalin A (1.25 μg/ml), or no antigen. The proliferative response was determined by measuring the incorporation of ^{3}[H]thymidine (1 μCi/well), which was added for the last 16 h of a 72-h culture.

ELISA for antibody detection. For this assay, 96-well flat-bottomed microtiter plates treated with glutaraldehyde (0.2%) were coated with 100 μl of p472 or A91 (10 μg/ml) in PBS, by overnight incubation at 4 °C. The plates were then blocked with PBS containing 1% bovine serum albumin (BSA) (Sigma, Petah Tikva, Israel) for 2 h at 37 °C, and washed with PBS containing Tween-20 (0.5%). Duplicate samples of the sera to be tested were added to the plates at different dilutions in PBS containing 1% BSA (1:500, 1:1000, 1:5,000, or 1:10,000). The plates were incubated for 2 h at 37 °C, then washed 5 times in PBS containing Tween-20 (0.5%). Peroxidase-conjugated AffiniPure goat anti-mouse IgG (Jackson ImmunoResearch, West Grove, PA, USA) or alkaline phosphatase goat anti-mouse IgM (Jackson), diluted 1:2500 in PBS, and 1% BSA were added, and the plates were incubated for 2 h at 37 °C. They were then washed 5 times in PBS containing Tween-20 (0.5%), and

alkaline phosphatase substrate solution (100 µl/well) was added. The reaction was stopped by the addition of 1 M phosphoric acid (100 µl/well). The plates were read with an ELISA plate reader (Anthos Labec Instruments, Salzburg, Austria) at 405 nm (in wells with anti-IgM antibody) or at 450 nm (in wells with anti-IgG antibody). Background OD was subtracted from the test OD values.

Assessment of recovery from spinal cord contusion. Behavioral recovery was scored in an open field using the Basso, Beattie, and Bresnahan locomotor rating scale (BBB), where a score of 0 registers complete paralysis and a score of 21 complete mobility (Basso et al., 1996). Blind scoring ensured that observers were not aware of the treatment received by each rat, as described (Hauben et al., 2001). Before each evaluation the rats were examined carefully for perineal infection, wounds in the hind limbs, and tail and foot autophagia.

Retrograde labeling of rubrospinal neurons. Four months after spinal cord contusion, rats were re-anesthetized and the dye rhodamine dextran amine (Fluoro-ruby, Molecular Probes, Eugene, OR, USA) was applied below the site of contusion at T12. After 5 days, the rats were again anesthetized and their brains were excised, processed, and cryosectioned. Sections taken through the red nucleus were inspected and analyzed qualitatively and quantitatively by fluorescence and confocal microscopy.

Partial crush injury of the rat optic nerve. The optic nerve was subjected to crush injury as previously described (Yoles et al., 2001).

Measurement of secondary degeneration of the rat optic nerve by retrograde labeling of retinal ganglion cells. Secondary degeneration of the optic nerve axons and their attached retinal ganglion cells (RGCs) was measured after post-injury application of the fluorescent lipophilic dye, 4-(4-(didecylamino)styryl)-N-methylpyridinium iodide (4-Di-10-Asp) (Molecular Probes Europe BV,

Netherlands), distally to the lesion site, 2 weeks after crush injury (Yoles and Schwartz, 1998).

Example 1. Posttraumatic vaccination with a Nogo-A-derived peptide reduces degeneration after incomplete spinal cord partial injury

The 18-amino-acid peptide (SYDSIKLEPENPPPYEEA) derived from Nogo-A (p472) was used it to vaccinate rats immediately after partial injury (contusion) of the spinal cord. The choice of peptide was based on previous observations that antibodies directed against p472 were as effective as the original IN-1 antibodies in blocking the myelin-inhibitory effect on neuronal growth, suggesting that this peptide might be the physiologically significant epitope within the Nogo-A protein (Chen et al., 2000; Raineteau et al., 2001; Merkler et al., 2001). Rats were injected with p472 or PBS emulsified in CFA. Fig. 1 shows the recovery of spinally contused female and male SPD rats after immunization with p472. Female rats immunized with p472 after injury recovered significantly better than females that were untreated or injected with PBS in CFA. The effect was discernible less than 2 weeks after immunization, and was stronger after 4 weeks (Fig. 1). The highest behavioral score (mean ± SEM) obtained in the p472-vaccinated group (n=5) was 8.0±0.8, compared to 3.4±0.9 in the untreated group (n=5). Control rats injected with PBS in CFA scored 5.5±0.5, which is higher than the untreated rats but still significantly lower than the p472-immunized rats. The above findings confirm that the endogenous immune response to a CNS insult can be boosted with myelin antigens and that the boosting has a beneficial effect (Yoles et al., 2001).

In all subsequent experiments, rats immunized with PBS in CFA were included as controls to verify that the effect induced by the peptide-specific immunization was more pronounced than the CFA-induced boosting of the endogenous response. In the males, improvement in recovery after p472 immunization was also significant, though less substantial than in the females: behavioral scores were 5.1±0.6 with p472 in CFA and 3.6±0.2 with PBS in CFA (Fig. 2A). The better recovery in the female rats is in line with a recent finding from

our laboratory (Hauben et al., 2001). These two experiments raised a key question: how does the observed effect of the Nogo-A-derived peptide in reducing post-traumatic immunization compare with that of MBP-derived peptides?

We recently reported that active post-traumatic vaccination of rats with an MBP-derived peptide (p87–99; A91) altered by one amino acid replacement (lysine 91 by alanine) leads to significant neuroprotection (Hauben et al., 2001). Here we compared the effects of A91 and p472 (both emulsified in CFA). The results obtained with the peptide A91 were better (Fig. 2B). The highest locomotor score obtained was 6.6±0.6 in rats immunized with A91 in CFA compared to 3.4±0.7 in rats injected with PBS in CFA. These results, and the small differences between control values in the two experiments (Fig. 2A vs. Fig. 2B), indicate that both peptides are effective, but do not permit definite conclusions about differences in their efficacy.

In a second set of experiments, SPD males with a milder contusive injury (caused by a weight drop from a height of 25 mm) were vaccinated with p472. As before, the effect was evident from day 14 on and then significant at all times of assessment, according to a two-tailed Student's *t*-test (Fig. 2C). The highest behavioral score in these rats was 8.9±0.5, compared to 7.0±0.45 after vaccination with PBS in CFA.

The behavioral recovery scores of p472-immunized rats after a weight drop from 25 mm reached plateau values that exceeded the threshold values correlated with sparing of the rubrospinal tracts needed for weight support. To confirm that the better recovery seen in these rats is related to a better survival of the rubrospinal tracts, we compared the integrity of the rubrospinal tracts in p472-immunized rats and rats injected with CFA in PBS (Fig. 2C). In rats with a BBB score around 8 (indicative of weight support), retrograde labeling gives an "all-or-none" indication of rubrospinal tract survival (Fehlings and Tator, 19951 Midha et al., 1987; Theriault and Tator, 1994). The examined control rats (average BBB score 6.5±0.5) showed

almost no labeled red nuclei. In the examined p472-immunized rats (BBB score 9.4±0.3; n=3) 142 ±46 red nuclei were labeled (Fig. 3).

To determine whether the immunity directed against Nogo-A (yielding the improved recovery observed here) was of cellular or humoral origin, we examined the immunized rats for specific anti-p472 T cells in lymph nodes and for antibodies in the serum. T cell proliferation assay revealed proliferating T cells in rats immunized with p472 in CFA when tested with the p472-derived peptide. The same lymphocytes were incubated with MBP-derived peptide (87–99) or OVA as controls. No p472-specific T cells were found in control rats injected with PBS in CFA (Fig. 4A). Antibody assays revealed no significant differences in IgM antibody titer between the rats injected with p472 in CFA and those injected with PBS in CFA (Fig. 4B).

None of the immunized rats developed symptoms of EAE. Since SPD rats are resistant to EAE induction, we examined whether p472 immunization of the EAE-susceptible Lewis rats would induce the disease. No clear signs of EAE were observed. It is possible, however, that infiltrating Nogo-specific T cells caused silent clinical changes in the CNS, as reported with other myelin-derived autoantigens, for example MAG (Weerth et al., 1999; Linington et al., 1993).

Example 2. T cells directed against Nogo-A-derived peptide are neuroprotective when passively transferred into SPD rats

The above results suggest that the neuroprotection observed after active vaccination with p472 is mediated by T cells specific to that peptide. To verify this suggestion we prepared a T cell line specific to p472. Passive transfer of anti-p472 T cells after spinal contusion in rats had a protective effect, which became apparent as early as 8 days after the injury; the highest behavioral score was 6±0.6 for rats treated with the T cells and 4±0.5 for untreated controls (Fig. 5).

The efficacy of different routes of immunization (active and passive; compare Fig. 2A and Fig. 5) should be compared with caution because the difference in their

numbers of available T cells at the lesion site during the therapeutic time window. Also, the efficacy of passive T-cell transfer is critically dependent on matching between the MHC-II characteristics of donor and recipient, meaning that inbred strains and homologous cells must be used. The SPD strain, though not inbred, is stably heterogeneous. It derives from a hybrid of Hooded males and Wistar females, with subsequent lines developed into a stable stock. The rats used in the present application were obtained from Harlan-Sprague-Dawley, and all were descended from the same original stock.

A primary T cell line was created by in-vitro propagation of lymphocytes from immunized SPD rats using antigen-presenting cells (APCs) from other SPD rats. Thus, despite the heterogeneity, the rats evidently share enough homology in their expressed MHC-II for this purpose (for the in-vitro propagation in this strain, we always used twice as many thymocytes as APCs than when working with inbred strains). Because of the heterogeneity, the passively transferred T cells might be less effective than in inbred rats using homologous cells; nevertheless, our results confirmed that the protection is mediated by T cells.

Example 3. Immunization with Nogo-A reduces loss of retinal ganglion cells after optic nerve injury

The post-traumatic loss of retinal ganglion cells (RGCs) can be significantly reduced by passive transfer of T cells directed against myelin antigens or by active immunization with these antigens. To determine whether active immunization is as effective with p472 as with other myelin antigens, SPD rats were immunized with p472 emulsified in CFA. Control injured rats were injected with PBS emulsified in CFA. When the rats were immunized prior to the insult, the number of surviving RGCs, determined by retrograde labeling 2 weeks after the insult, was significantly larger in the p472-immunized rats than in the controls (Fig. 6A). Passive transfer of p472-specific T cells immediately after the insult had a beneficial effect on recovery, like that seen in our studies with T cells that are specific to myelin peptides or cross-reactive with Cop-1 (Hauben et al., 2000a; Kipnis et al., 2000). Active immunization

with A91 immediately after optic nerve injury (Fig. 6B), like passive transfer of the T cells specific to p472 (Fig. 6A), yielded a better RGC survival rate than that seen in the control (Fig. 6A, B). This finding suggests that that both p472 and A91 are potentially neuroprotective antigens, and that the possible difference in their immunogenicity is manifested by how promptly they induce a response. No such difference is observed when the T cells are passively transferred.

Discussion

Recovery from spinal cord injury depends on the severity of the insult (location of injury and number of spared neurons), the amount of secondary degeneration, the ability to regenerate, and the ability to activate local mechanisms of plasticity. Whereas the question of regeneration is still largely unsolved, much more is now known about neuroprotection in general, especially in the context of the spinal cord. In particular, it is now clear why neuronal degeneration continues to progress after the primary insult, and how the damage is propagated.

The design of therapeutic strategies has been hampered by some apparent contradictions in the findings of research, especially concerning the question of whether the recovery process is advanced or impeded by inflammation. Anti-inflammatory drugs were reported to be beneficial after spinal cord injury, leading to the tentative conclusion that an inflammatory reaction is bad for recovery. However, recent studies (Yoles et al., 2001; Serpe et al., 1999 and 2000) indicate that the adaptive immune response, which is spontaneously evoked by a spinal cord injury, is beneficial for recovery in some strains, depending on their inherent susceptibility to EAE (Kipnis et al., 2001). The T cell-dependent protective autoimmunity was also found to be characterized by sexual dimorphism, with female rats recovering better than males after spinal cord contusion (Hauben et al, unpublished results). The injury-evoked, T cell-mediated protective response was found to be boosted in EAE-resistant strains (or induced in EAE-susceptible strains) by passive transfer of anti-MBP T cells or by active vaccination with certain myelin-associated self-antigens (Hauben et al., 2001).

According to the present invention, it is shown that a peptide derived from Nogo-A, a myelin component known to inhibit post-traumatic regeneration, can activate the immune system in a beneficial way. This peptide had a neuroprotective effect in the same model and with the same therapeutic time window as that shown by us to accommodate a T cell-based therapy (Hauben et al., 2000a). Thus, after partial crush injury of the rat optic nerve or spinal cord, prompt vaccination (within 1 h after the injury) with Nogo-A-derived peptide emulsified in CFA evoked a T cell-mediated protective autoimmunity. Within this time window we detected p472-specific T cells in the immunized injured rats, but no anti-p472 antibodies. Similar results were obtained after passive transfer of T cells specific to the Nogo-A-derived peptide. We therefore considered it important to determine whether a peptide derived from Nogo-A has advantages over other myelin-associated peptides as a potential vaccine. Based on the present comparison with the effect of an MBP-derived peptide, the answer seems to be that both peptides are potential candidates.

Recent studies reveal that, for protective autoimmunity, both autoimmune T cells and regulatory T cells are essential constituents of this network, but neither of them on its own suffices to bring about neuroprotection (Schwartz and Kipnis, 2001). When considering the use of a particular myelin-associated peptide for therapy, it is important to ascertain that the T cell response it elicits falls within the therapeutic window, and that it will confer the desired benefit without risking the development of an autoimmune disease. Also, such peptides should not elicit any immune activity that interferes with their essential physiological activities, if any. In susceptible rat strains, a modified encephalitogenic peptide was found to be beneficial (Hauben et al., 2001). The present invention demonstrates that in EAE-susceptible rat strains, Nogo-A-derived peptides were not encephalitogenic. However, we cannot rule out the possibility that vaccination with Nogo-A-derived peptides might evoke, in addition to the observed T cell response, a delayed antibody response. Though apparently not needed for neuroprotection, these antibodies might interfere with the essential activities of Nogo-A in maintaining the integrity of the adult CNS. Such interference might be a potential side effect of the use of anti-

Nogo-A antibodies for regeneration (Raineteau et al., 2001). In view of this possible drawback, it might be preferable to choose a myelin-associated peptide that does not play a role in neuronal plasticity. Alternatively, it might be possible to exploit the neuroprotective property of Nogo-derived peptides in a way that avoids the appearance of antibodies, by using passive vaccination with anti-Nogo T cells rather than active immunization with the peptides themselves.

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CLAIMS:

1. Use of an agent selected from: (a) a peptide derived from a Nogo or a Nogo receptor molecule and (b) autologous T cells recognizing a peptide of (a) in the context of class II MHC molecules, for the manufacture of a pharmaceutical composition which elicits a T-cell-mediated neuroprotective response.

2. Use according to claim 1 wherein said peptide is selected from:

(i) a peptide having a sequence comprised within the sequence of a Nogo or a Nogo receptor molecule; and

(ii) a peptide of (i) modified by replacement of one or more amino acid residues by different amino acid residues at the T cell receptor (TCR) binding site and not at the MHC binding site(s), so that the immune response is activated but not anergized.

3. Use according to claim 1 or 2 wherein the peptide is derived from a mammalian Nogo molecule.

4. Use according to claim 3 wherein the Nogo molecule is selected from rat Nogo-A (SEQ ID NO:1), rat Nogo-B (SEQ ID NO:3), rat Nogo-C (SEQ ID NO:4), human Nogo-A (SEQ ID NO:5), human Nogo-B (SEQ ID NO:6), human Nogo-C (SEQ ID NO:7).

5. Use according to claim 4 wherein the Nogo molecule is Nogo-A (SEQ ID NO:1).

6. Use according to claim 5 wherein the peptide is derived from the rat Nogo-A and has the sequence:

S Y D S I K L E P E N P P P Y E E A (SEQ ID NO:2).

7. Use according to claim 6 wherein the peptide of SEQ ID NO:2 is modified by deletion, addition or replacement of one or more amino acids.

8. Use according to claim 1 or 2 wherein the peptide is derived from a Nogo receptor molecule.

9. Use according to claim 5 wherein the Nogo receptor-derived peptide is selected from the sequences:

S G V P S N L P Q R L A G R D (SEQ ID NO:10)

T R S H C R L G Q A G S G S S (SEQ ID NO:11)

10. Use according to claim 9 wherein the peptide of SEQ ID NO:10 or SEQ ID NO:11 is modified by deletion, addition or replacement of one or more amino acids.

11. Use according to any one of claims 1 to 10 wherein the composition contains at least one adjuvant that induces a Th-1 response to the peptide.

12. Use according to claim 11 wherein said at least one adjuvant is selected from lipopolysaccharide (LPS), a synthetic oligodeoxynucleotide containing CpG motifs, a killed bacterium such as Brucella abortus, Mycobacterium vaccae or BCG, a bacterial toxin such as tetanus toxoid, Pseudomonas aeruginosa toxin A or Bordetella pertussis toxin, beta-galactosidase, influenza virus hemagglutinin and nucleoprotein, hepatitis B core and surface antigens, block copolymer adjuvant P1005, Algammulin and gamma-inulin.

13. Use according to any one of claims 1 to 10 wherein the peptide is covalently associated with a lipopeptidic immunostimulant, encapsidated into liposomes or is admixed with controlled-release microparticles or with a immune-stimulating complex (iscom) matrix.

14. Use according to claim 1 wherein the agent is autologous T cells which recognize the peptide of (a) in the context of class II MHC molecules.

15. Use according to claim 14 wherein said autologous T cells are derived from stored T cells.

16. Use according to any one of claims 1-15 for neuroprotection in the CNS or for protecting CNS cells from glutamate toxicity in an area of myelinated axons.

17. Use according to claim 16, for treating an injury, disorder or disease of the CNS in order to prevent or inhibit neuronal degeneration or for treating an injury, disorder or disease in an area of myelinated axons of the CNS caused or exacerbated by glutamate toxicity,

18. Use according to claim 17, wherein said injury, disorder or disease is spinal cord injury or brain injury, blunt trauma, penetrating trauma, brain coup or contrecoup, hemorrhagic stroke, or ischemic stroke.

19. Use according to claim 18, wherein said injury is spinal cord injury.

20. Use according to claim 20, wherein said injury, disorder or disease is a senile dementia including Alzheimer's disease, Lewy Body disease and multi-infarct dementia, a Parkinsonian syndrome including Parkinson's disease, facial nerve (Bell's) palsy, Huntington's disease, a motor neuron disease including amyotrophic lateral sclerosis, a prion disease including Creutzfeldt-Jakob disease, Alper's disease, Batten disease, Cockayne syndrome, , status epilepticus, epilepsy, multiple sclerosis, amnesia, seizures, oxidative stress, opiate tolerance and dependence, and glaucoma.

21. Use according to claim 20, wherein said injury, disorder or disease is glaucoma.

22. A pharmaceutical composition which elicits a T-cell-mediated neuroprotective response comprising a pharmaceutically acceptable carrier and an agent selected from: (a) a peptide derived from a Nogo or a Nogo receptor molecule and (b) autologous T cells recognizing a peptide of (a) in the context of class II MHC molecules.

23. A pharmaceutical composition according to claim 22 wherein said peptide is selected from:

(i) a peptide having a sequence comprised within the sequence of a Nogo or a Nogo receptor molecule; and

(ii) a peptide of (i) modified by replacement of one or more amino acid residues by different amino acid residues at the T cell receptor (TCR) binding site and not at the MHC binding site(s), so that the immune response is activated but not anergized.

24. A pharmaceutical composition according to claim 22 or 23 wherein the peptide is derived from a mammalian Nogo molecule.

25. A pharmaceutical composition according to claim 24 wherein the Nogo molecule is selected from rat Nogo-A (SEQ ID NO:1), rat Nogo-B (SEQ ID NO:3), rat Nogo-C (SEQ ID NO:4), human Nogo-A (SEQ ID NO:5), human Nogo-B (SEQ ID NO:6), human Nogo-C (SEQ ID NO:7).

26. A pharmaceutical composition according to claim 25 wherein the Nogo molecule is Nogo-A (SEQ ID NO:1).

27. A pharmaceutical composition according to claim 26 wherein the peptide is derived from the rat Nogo-A and has the sequence:

S Y D S I K L E P E N P P P Y E E A (SEQ ID NO:2).

28. A pharmaceutical composition according to claim 27 wherein the peptide of SEQ ID NO:2 is modified by deletion, addition or replacement of one or more amino acids.

29. A pharmaceutical composition according to claim 22 or 23 wherein the peptide is derived from a Nogo receptor molecule.

30. A pharmaceutical composition according to claim 29 wherein the Nogo receptor-derived peptide is selected from the sequences:

S G V P S N L P Q R L A G R D (SEQ ID NO:10)

T R S H C R L G Q A G S G S S (SEQ ID NO:11)

31. A pharmaceutical composition according to claim 30 wherein the peptide of SEQ ID NO:10 or SEQ ID NO:11 is modified by deletion, addition or replacement of one or more amino acids.

32. A pharmaceutical composition according to any one of claims 22 to 31 wherein the composition contains at least one adjuvant that induces a Th-1 response to the peptide.

33. A pharmaceutical composition according to claim 32 wherein said at least one adjuvant is selected from lipopolysaccharide (LPS), a synthetic oligodeoxynucleotide containing CpG motifs, a killed bacterium such as Brucella abortus, Mycobacterium vaccae or BCG, a bacterial toxin such as tetanus toxoid, Pseudomonas aeruginosa toxin A or Bordetella pertussis toxin, beta-galactosidase, influenza virus hemagglutinin and nucleoprotein, hepatitis B core and surface antigens, block copolymer adjuvant P1005, Algammulin and gamma-inulin.

34. A pharmaceutical composition according to any one of claims 22 to 31 wherein the peptide is covalently associated with a lipopeptidic immunostimulant,

encapsidated into liposomes or is admixed with controlled-release microparticles or with a immune-stimulating complex (iscom) matrix.

35. A pharmaceutical composition according to claim 22 wherein the agent is autologous T cells which recognize the peptide of (a) in the context of class II MHC molecules.

36. A pharmaceutical composition according to claim 35 wherein said autologous T cells are derived from stored T cells.

37. A pharmaceutical composition according to any one of claims 22-36 for neuroprotection in the CNS or for protecting CNS cells from glutamate toxicity in an area of myelinated axons.

38. A pharmaceutical composition according to claim 37, for treating an injury, disorder or disease of the CNS in order to prevent or inhibit neuronal degeneration or for treating an injury, disorder or disease in an area of myelinated axons of the CNS caused or exacerbated by glutamate toxicity.

39. A pharmaceutical composition according to claim 38, wherein said injury, disorder or disease is spinal cord injury or brain injury, blunt trauma, penetrating trauma, brain coup or contrecoup, hemorrhagic stroke, or ischemic stroke.

40. A pharmaceutical composition according to claim 39, wherein said injury is spinal cord injury.

41. A pharmaceutical composition according to claim 38, wherein said injury, disorder or disease is a senile dementia including Alzheimer's disease, Lewy Body disease and multi-infarct dementia, Parkinsonian syndrome including Parkinson's disease, facial nerve (Bell's) palsy, Huntington's disease, a motor neuron disease

including amyotrophic lateral sclerosis, a prion disease including Creutzfeldt-Jakob disease, Alper's disease, Batten disease, Cockayne syndrome, status epilepticus, epilepsy, multiple sclerosis, amnesia, seizures, oxidative stress, opiate tolerance and dependence, and glaucoma.

42. A pharmaceutical composition according to claim 41, wherein said injury, disorder or disease is glaucoma.

43. A method for conferring neuroprotection and thus preventing or inhibiting neuronal degeneration in the central nervous system for ameliorating the effects of injury, disorder or disease, comprising administering to an individual in need at least one agent selected from the group consisting of: (a) a peptide derived from a Nogo or a Nogo receptor molecule and (b) autologous T cells recognizing a peptide of (a) in the context of class II MHC molecules.

44. A method according to claim 43 wherein said peptide is selected from the group consisting of:

(i) a peptide having a sequence comprised within the sequence of a Nogo or a Nogo receptor molecule; and

(ii) a peptide of (i) modified by replacement of one or more amino acid residues by different amino acid residues at the T cell receptor (TCR) binding site and not at the MHC binding site(s), so that the immune response is activated but not anergized.

45. A method according to claim 43 or 44 wherein the peptide is derived from a mammalian Nogo molecule.

46. A method according to claim 45 wherein the Nogo molecule is selected from rat Nogo-A (SEQ ID NO:1), rat Nogo-B (SEQ ID NO:3), rat Nogo-C (SEQ ID NO:4),

human Nogo-A (SEQ ID NO:5), human Nogo-B (SEQ ID NO:6), human Nogo-C (SEQ ID NO:7).

47. A method according to claim 46 wherein the Nogo molecule is Nogo-A (SEQ ID NO:1).

48. A method according to claim 47 wherein the peptide is derived from the rat Nogo-A and has the sequence:

S Y D S I K L E P E N P P P Y E E A (SEQ ID NO:2).

49. A method according to claim 48 wherein the peptide of SEQ ID NO:2 is modified by deletion, addition or replacement of one or more amino acids.

50. A method according to claim 43 or 44 wherein the peptide is derived from a Nogo receptor molecule.

51. A method according to claim 51 wherein the Nogo receptor-derived peptide is selected from the sequences consisting of:

S G V P S N L P Q R L A G R D (SEQ ID NO:10)

T R S H C R L G Q A G S G S S (SEQ ID NO:11)

52. A method according to claim 51 wherein the peptide of SEQ ID NO:10 or SEQ ID NO:11 is modified by deletion, addition or replacement of one or more amino acids.

53. A method according to claim 43 wherein the peptide is administered together with at least one adjuvant that induces a Th-1 response to the peptide.

54. A method according to claim 53 wherein said at least one adjuvant is selected from the group consisting of: lipopolysaccharide (LPS), a synthetic oligodeoxy-

nucleotide containing CpG motifs, a killed bacterium such as Brucella abortus, Mycobacterium vaccae or BCG, a bacterial toxin such as tetanus toxoid, Pseudomonas aeruginosa toxin A or Bordetella pertussis toxin, beta-galactosidase, influenza virus hemagglutinin and nucleoprotein, hepatitis B core and surface antigens, block copolymer adjuvant P1005, Algammulin and gamma-inulin.

55. A method according to claim 43 wherein the peptide is covalently associated with a lipopeptidic immunostimulant, encapsidated into liposomes or is admixed with controlled-release microparticles or with a immune-stimulating complex (iscom) matrix.

56. A method according to claim 43 wherein the agent is autologous T cells which recognize the peptide of (a) in the context of class II MHC molecules.

57. A method according to claim 56 wherein said autologous T cells are derived from stored T cells.

58. A method according to claim 43 which comprises administering to an individual in need a peptide derived from a Nogo or a Nogo receptor molecule followed by administration of T cells which recognize said peptide in the context of class II MHC molecules.

59. A method according to claim 43 for neuroprotection in the CNS or for protecting CNS cells from glutamate toxicity in an area of myelinated axons.

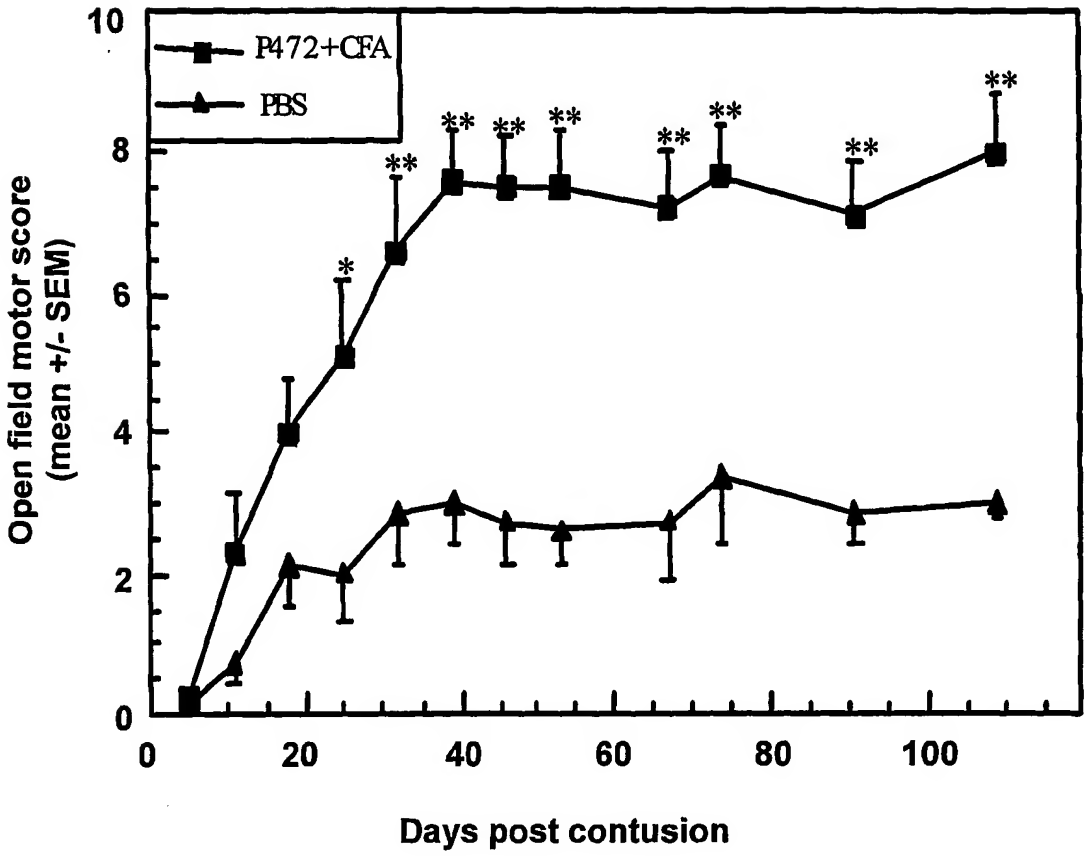
60. A method according to claim 43, wherein said injury, disorder or disease is spinal cord injury or brain injury, blunt trauma, penetrating trauma, brain coup or contrecoup, hemorrhagic stroke, or ischemic stroke.

61. A method according to claim 60 wherein said injury is spinal cord injury.

62. A method according to claim 43, wherein said injury, disorder or disease is selected from the group consisting of a senile dementia including Alzheimer's disease, Lewy Body disease and multi-infarct dementia, a Parkinsonian syndrome including Parkinson's disease, facial nerve (Bell's) palsy, Huntington's disease, a motor neuron disease including amyotrophic lateral sclerosis, a prion disease including Creutzfeldt-Jakob disease, Alper's disease, Batten disease, Cockayne syndrome, status epilepticus, epilepsy, multiple sclerosis, amnesia, seizures, oxidative stress, opiate tolerance and dependence, and glaucoma.

63. A method according to claim 62, wherein said injury, disorder or disease is glaucoma.

Fig. 1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*
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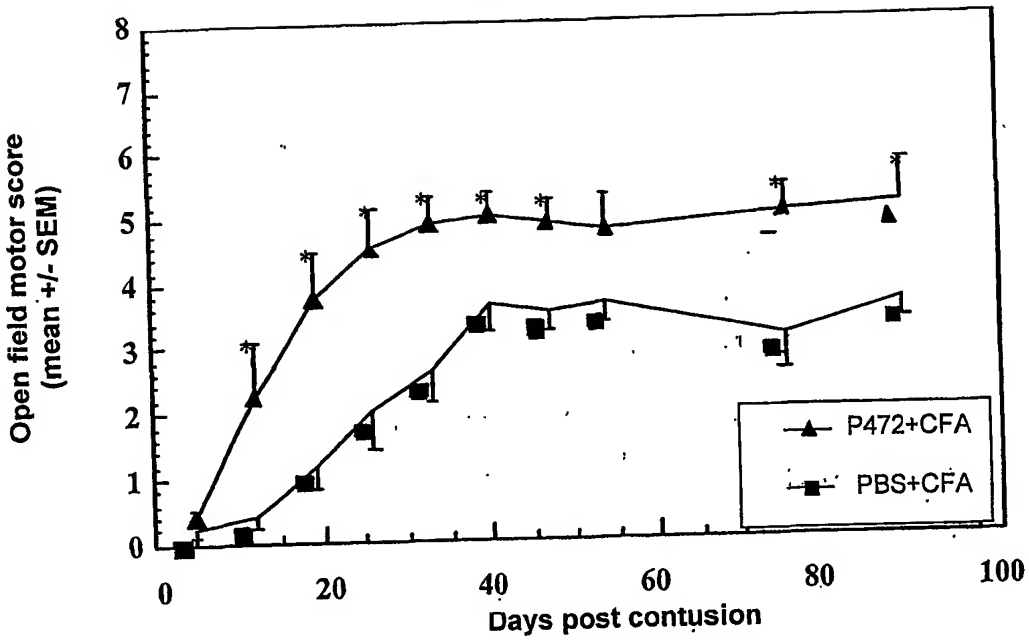
Fig. 2A


Open field motor score
(mean +/- SEM)
Days post contusion
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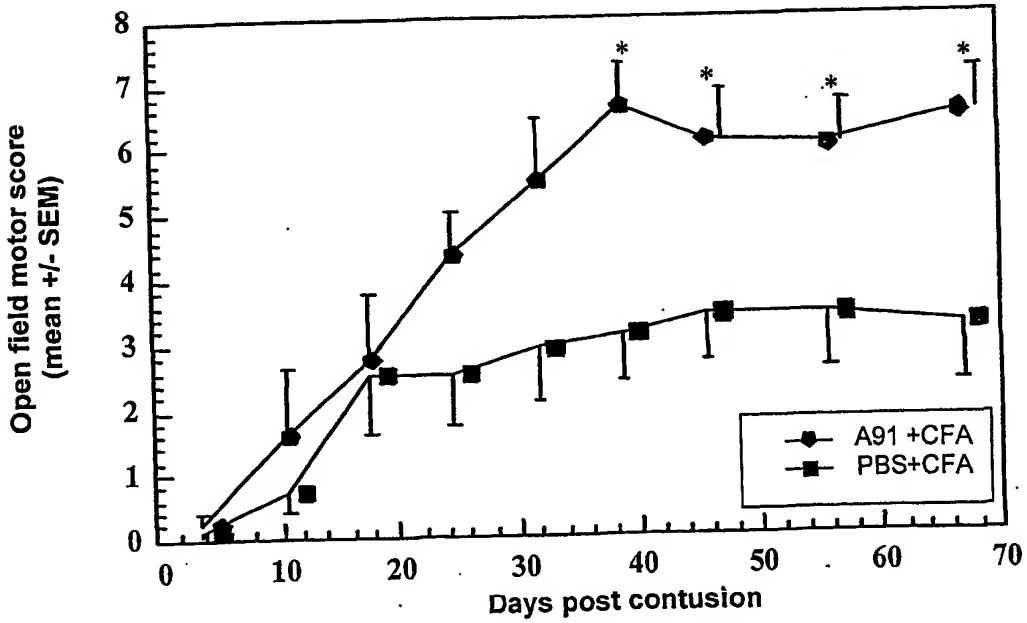
20
40
60
80
100

Fig. 2B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(mean +/- SEM)
Days post contusion
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PBS+CFA
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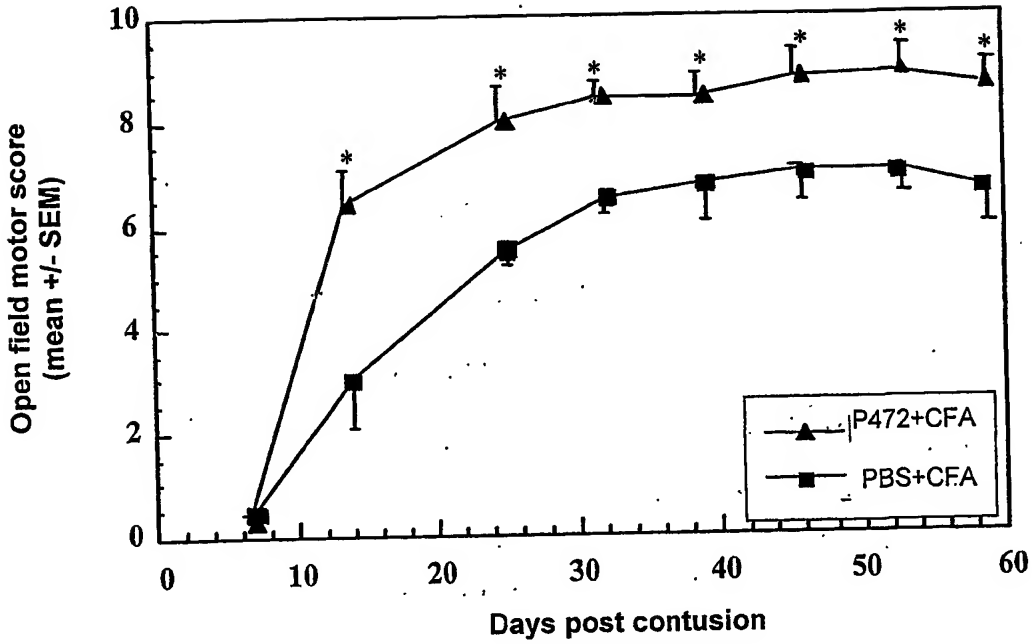
50
60
70

Fig. 2C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Days post contusion
P472+CFA
PBS+CFA
*

Fig. 3A

Fig. 3B

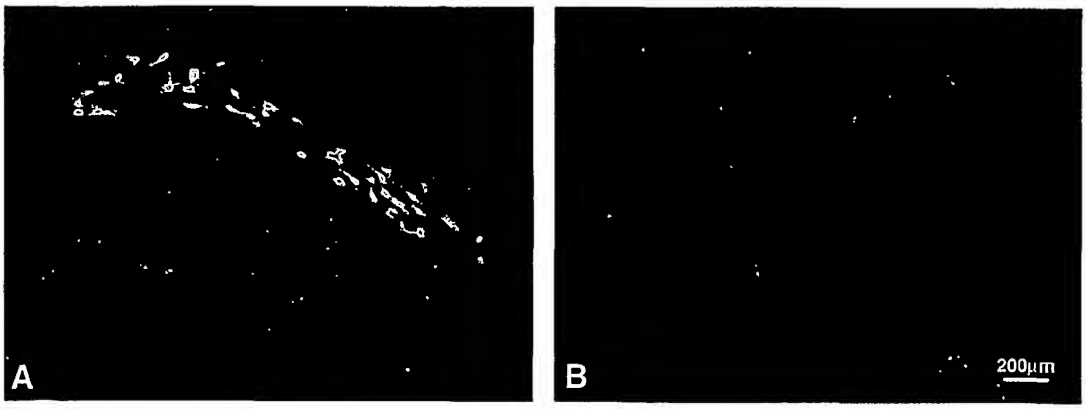
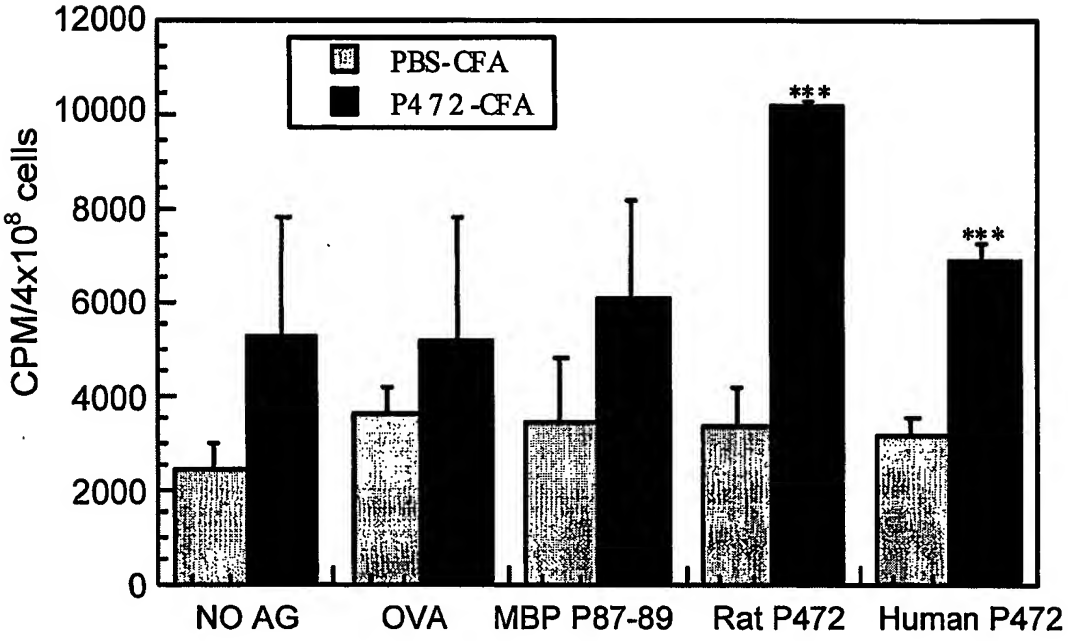


Fig. 4A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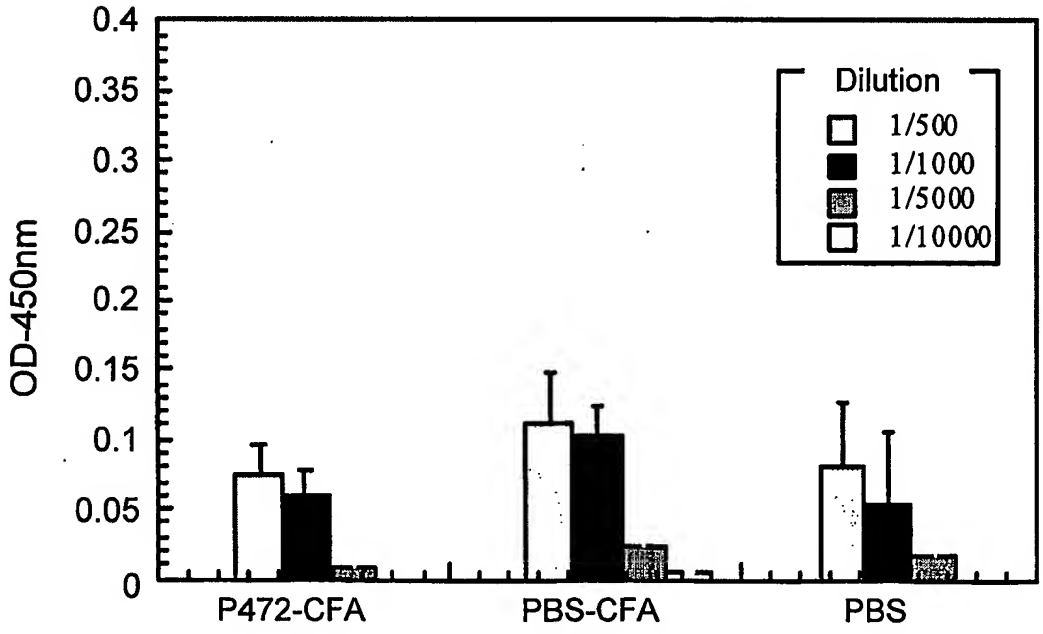
OVA
MBP P87-89
Rat P472
Human P472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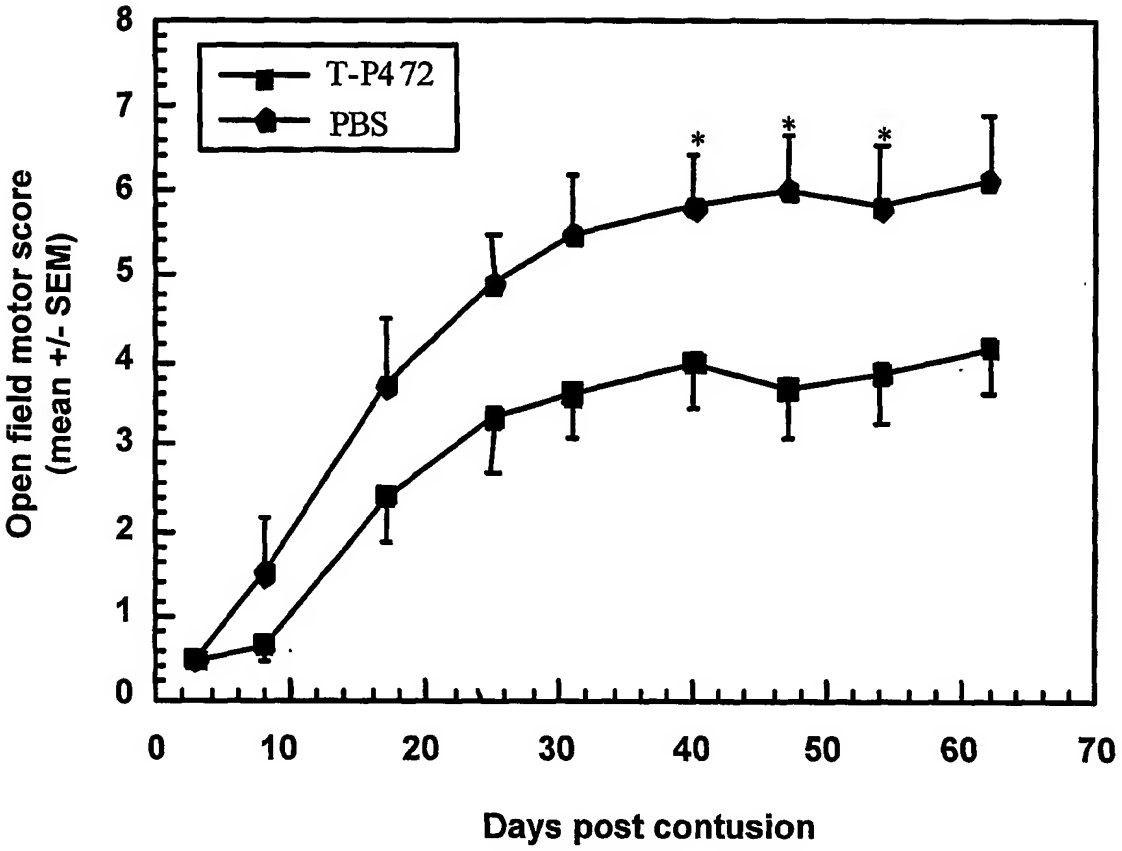
PBS-CFA
PBS

Fig. 4B

Fig. 5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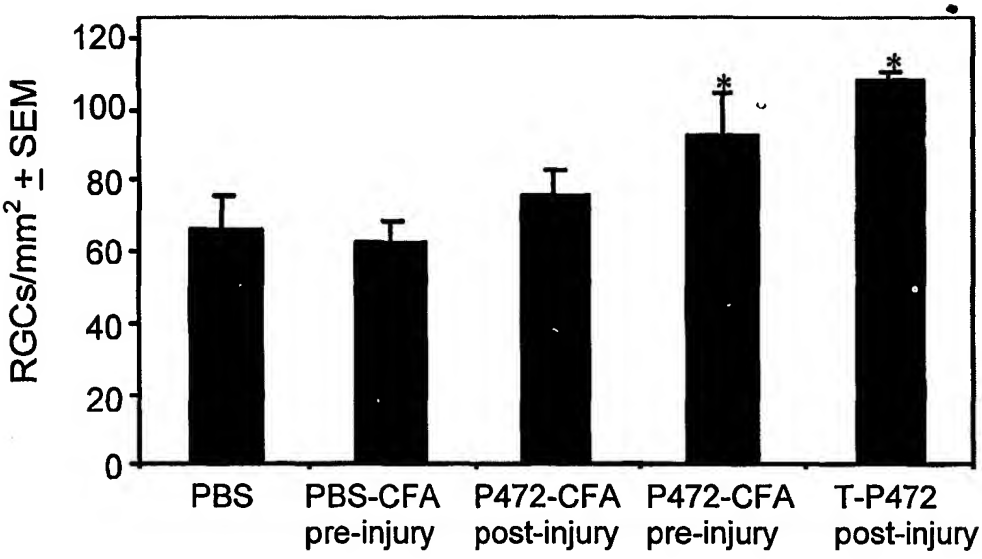
*
*
*

Fig. 6A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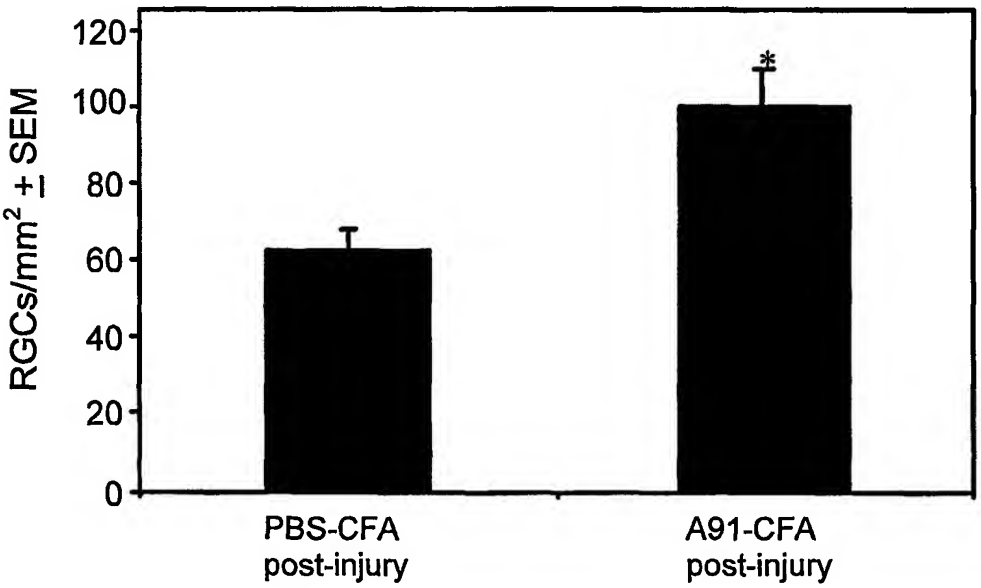
P472-CFA post-injury
P472-CFA pre-injury
T-P472 post-injury


RGCs/mm^2 ± SEM
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A91-CFA post-injury

Fig. 6B

SEQUENCE LISTING

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<120> NOGO AND NOGO RECEPTOR DERIVED PEPTIDES FOR T-CELL MEDIATED NEUROPROTECTION

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<150> US 09/893,348
<151> 2001-06-28

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<170> PatentIn version 3.1

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        35                  40                  45

Tyr Ile Ala Leu Ala Leu Leu Ser Val Thr Ile Ser Phe Arg Ile Tyr
    50                  55                  60

Lys Gly Val Ile Gln Ala Ile Gln Lys Ser Asp Glu Gly His Pro Phe
```

65 70 75 80

Arg Ala Tyr Leu Glu Ser Glu Val Ala Ile Ser Glu Glu Leu Val Gln
85 90 95

Lys Tyr Ser Asn Ser Ala Leu Gly His Val Asn Ser Thr Ile Lys Glu
100 105 110

Leu Arg Arg Leu Phe Leu Val Asp Asp Leu Val Asp Ser Leu Lys Phe
115 120 125

Ala Val Leu Met Trp Val Phe Thr Tyr Val Gly Ala Leu Phe Asn Gly
130 135 140

Leu Thr Leu Leu Ile Leu Ala Leu Ala Leu Ile Ser Leu Phe Ser Ile Pro Val
145 150 155 160

Ile Tyr Glu Arg His Gln Val Gln Ile Asp His Tyr Leu Gly Leu Ala
165 170 175

Asn Lys Ser Val Lys Asp Ala Met Ala Lys Ile Gln Ala Lys Ile Pro
180 185 190

Gly Leu Lys Arg Lys Ala Asp
195

<210> 5
<211> 1192
<212> PRT
<213> Homo sapiens

<400> 5

Met Glu Asp Leu Asp Gln Ser Pro Leu Val Ser Ser Ser Asp Ser Pro
1 5 10 15

Pro Arg Pro Gln Pro Ala Phe Lys Tyr Gln Phe Val Arg Glu Pro Glu
20 25 30

Asp Glu Glu Glu Glu Glu Glu Glu Glu Glu Glu Asp Glu Asp Glu Asp
35 40 45

Leu Glu Glu Leu Glu Val Leu Glu Arg Lys Pro Ala Ala Gly Leu Ser
50 55 60

Ala Ala Pro Val Pro Thr Ala Pro Ala Ala Gly Ala Pro Leu Met Asp
65 70 75 80

Phe Gly Asn Asp Phe Val Pro Pro Ala Pro Arg Gly Pro Leu Pro Ala
85 90 95

Ala Pro Pro Val Ala Pro Glu Arg Gln Pro Ser Trp Asp Pro Ser Pro
100 105 110

Val Ser Ser Thr Val Pro Ala Pro Ser Pro Leu Ser Ala Ala Ala Val
115 120 125

Ser Pro Ser Lys Leu Pro Glu Asp Asp Glu Pro Pro Ala Arg Pro Pro
130 135 140

Pro Pro Pro Pro Ala Ser Val Ser Pro Gln Ala Glu Pro Val Trp Thr
145 150 155 160

Pro Pro Ala Pro Ala Pro Ala Ala Pro Pro Ser Thr Pro Ala Ala Pro
165 170 175

Lys Arg Arg Gly Ser Ser Gly Ser Val Asp Glu Thr Leu Phe Ala Leu
180 185 190

Pro Ala Ala Ser Glu Pro Val Ile Arg Ser Ser Ala Glu Asn Met Asp
195 200 205

Leu Lys Glu Gln Pro Gly Asn Thr Ile Ser Ala Gly Gln Glu Asp Phe
210 215 220

Pro Ser Val Leu Leu Glu Thr Ala Ala Ser Leu Pro Ser Leu Ser Pro
225 230 235 240

Leu Ser Ala Ala Ser Phe Lys Glu His Glu Tyr Leu Gly Asn Leu Ser
245 250 255

Thr Val Leu Pro Thr Glu Gly Thr Leu Gln Glu Asn Val Ser Glu Ala
260 265 270

Ser Lys Glu Val Ser Glu Lys Ala Lys Thr Leu Leu Ile Asp Arg Asp
275 280 285

Leu Thr Glu Phe Ser Glu Leu Glu Tyr Ser Glu Met Gly Ser Ser Phe
290 295 300

Ser Val Ser Pro Lys Ala Glu Ser Ala Val Ile Val Ala Asn Pro Arg
305 310 315 320

Glu Glu Ile Ile Val Lys Asn Lys Asp Glu Glu Glu Lys Leu Val Ser
325 330 335

Asn Asn Ile Leu His Asn Gln Gln Glu Leu Pro Thr Ala Leu Thr Lys
340 345 350

Leu Val Lys Glu Asp Glu Val Val Ser Ser Glu Lys Ala Lys Asp Ser
355 360 365

Phe Asn Glu Lys Arg Val Ala Val Glu Ala Pro Met Arg Glu Glu Tyr
370 375 380

Ala Asp Phe Lys Pro Phe Glu Arg Val Trp Glu Val Lys Asp Ser Lys
385 390 395 400

Glu Asp Ser Asp Met Leu Ala Ala Gly Gly Lys Ile Glu Ser Asn Leu
405 410 415

Glu Ser Lys Val Asp Lys Lys Cys Phe Ala Asp Ser Leu Glu Gln Thr

420 425 430

Asn His Glu Lys Asp Ser Glu Ser Ser Asn Asp Asp Thr Ser Phe Pro
435 440 445

Ser Thr Pro Glu Gly Ile Lys Asp Arg Pro Gly Ala Tyr Ile Thr Cys
450 455 460

Ala Pro Phe Asn Pro Ala Ala Thr Glu Ser Ile Ala Thr Asn Ile Phe
465 470 475 480

Pro Leu Leu Gly Asp Pro Thr Ser Glu Asn Lys Thr Asp Glu Lys Lys
485 490 495

Ile Glu Glu Lys Lys Ala Gln Ile Val Thr Glu Lys Asn Thr Ser Thr
500 505 510

Lys Thr Ser Asn Pro Phe Leu Val Ala Ala Gln Asp Ser Glu Thr Asp
515 520 525

Tyr Val Thr Thr Asp Asn Leu Thr Lys Val Thr Glu Glu Val Val Ala
530 535 540

Asn Met Pro Glu Gly Leu Thr Pro Asp Leu Val Gln Glu Ala Cys Glu
545 550 555 560

Ser Glu Leu Asn Glu Val Thr Gly Thr Lys Ile Ala Tyr Glu Thr Lys
565 570 575

Met Asp Leu Val Gln Thr Ser Glu Val Met Gln Glu Ser Leu Tyr Pro
580 585 590

Ala Ala Gln Leu Cys Pro Ser Phe Glu Glu Ser Glu Ala Thr Pro Ser
595 600 605

Pro Val Leu Pro Asp Ile Val Met Glu Ala Pro Leu Asn Ser Ala Val
610 615 620

Pro Ser Ala Gly Ala Ser Val Ile Gln Pro Ser Ser Ser Pro Leu Glu
625 630 635 640

Ala Ser Ser Val Asn Tyr Glu Ser Ile Lys His Glu Pro Glu Asn Pro
645 650 655

```
Pro Pro Tyr Glu Glu Ala Met Ser Val Ser Leu Lys Lys Val Ser Gly
            660                 665                 670

Ile Lys Glu Glu Ile Lys Glu Pro Glu Asn Ile Asn Ala Ala Leu Gln
        675                 680                 685

Glu Thr Glu Ala Pro Tyr Ile Ser Ile Ala Cys Asp Leu Ile Lys Glu
    690                 695                 700

Thr Lys Leu Ser Ala Glu Pro Ala Pro Asp Phe Ser Asp Tyr Ser Glu
705                 710                 715                 720

Met Ala Lys Val Glu Gln Pro Val Pro Asp His Ser Glu Leu Val Glu
                725                 730                 735

Asp Ser Ser Pro Asp Ser Glu Pro Val Asp Leu Phe Ser Asp Asp Ser
            740                 745                 750

Ile Pro Asp Val Pro Gln Lys Gln Asp Glu Thr Val Met Leu Val Lys
        755                 760                 765

Glu Ser Leu Thr Glu Thr Ser Phe Glu Ser Met Ile Glu Tyr Glu Asn
    770                 775                 780

Lys Glu Lys Leu Ser Ala Leu Pro Pro Glu Gly Gly Lys Pro Tyr Leu
785                 790                 795                 800

Glu Ser Phe Lys Leu Ser Leu Asp Asn Thr Lys Asp Thr Leu Leu Pro
                805                 810                 815

Asp Glu Val Ser Thr Leu Ser Lys Lys Glu Lys Ile Pro Leu Gln Met
            820                 825                 830

Glu Glu Leu Ser Thr Ala Val Tyr Ser Asn Asp Asp Leu Phe Ile Ser
        835                 840                 845

Lys Glu Ala Gln Ile Arg Glu Thr Glu Thr Phe Ser Asp Ser Ser Pro
    850                 855                 860

Ile Glu Ile Ile Asp Glu Phe Pro Thr Leu Ile Ser Ser Lys Thr Asp
865                 870                 875                 880

Ser Phe Ser Lys Leu Ala Arg Glu Tyr Thr Asp Leu Glu Val Ser His
```

885 890 895

Lys Ser Glu Ile Ala Asn Ala Pro Asp Gly Ala Gly Ser Leu Pro Cys
900 905 910

Thr Glu Leu Pro His Asp Leu Ser Leu Lys Asn Ile Gln Pro Lys Val
915 920 925

Glu Glu Lys Ile Ser Phe Ser Asp Asp Phe Ser Lys Asn Gly Ser Ala
930 935 940

Thr Ser Lys Val Leu Leu Leu Pro Pro Asp Val Ser Ala Leu Ala Thr
945 950 955 960

Gln Ala Glu Ile Glu Ser Ile Val Lys Pro Lys Val Leu Val Lys Glu
965 970 975

Ala Glu Lys Lys Leu Pro Ser Asp Thr Glu Lys Glu Asp Arg Ser Pro
980 985 990

Ser Ala Ile Phe Ser Ala Glu Leu Ser Lys Thr Ser Val Val Asp Leu
995 1000 1005

Leu Tyr Trp Arg Asp Ile Lys Lys Thr Gly Val Val Phe Gly Ala
1010 1015 1020

Ser Leu Phe Leu Leu Leu Ser Leu Thr Val Phe Ser Ile Val Ser
1025 1030 1035

Val Thr Ala Tyr Ile Ala Leu Ala Leu Leu Ser Val Thr Ile Ser
1040 1045 1050

Phe Arg Ile Tyr Lys Gly Val Ile Gln Ala Ile Gln Lys Ser Asp
1055 1060 1065

Glu Gly His Pro Phe Arg Ala Tyr Leu Glu Ser Glu Val Ala Ile
1070 1075 1080

Ser Glu Glu Leu Val Gln Lys Tyr Ser Asn Ser Ala Leu Gly His
1085 1090 1095

Val Asn Cys Thr Ile Lys Glu Leu Arg Arg Leu Phe Leu Val Asp
1100 1105 1110

Asp Leu Val Asp Ser Leu Lys Phe Ala Val Leu Met Trp Val Phe
1115 1120 1125

Thr Tyr Val Gly Ala Leu Phe Asn Gly Leu Thr Leu Leu Ile Leu
1130 1135 1140

Ala Leu Ile Ser Leu Phe Ser Val Pro Val Ile Tyr Glu Arg His
1145 1150 1155

Gln Ala Gln Ile Asp His Tyr Leu Gly Leu Ala Asn Lys Asn Val
1160 1165 1170

Lys Asp Ala Met Ala Lys Ile Gln Ala Lys Ile Pro Gly Leu Lys
1175 1180 1185

Arg Lys Ala Glu
1190

<210> 6
<211> 373
<212> PRT
<213> Homo sapiens

<400> 6

Met Glu Asp Leu Asp Gln Ser Pro Leu Val Ser Ser Ser Asp Ser Pro
1 5 10 15

Pro Arg Pro Gln Pro Ala Phe Lys Tyr Gln Phe Val Arg Glu Pro Glu
20 25 30

Asp Glu Glu Glu Glu Glu Glu Glu Glu Glu Asp Glu Asp Glu Asp
35 40 45

Leu Glu Glu Leu Glu Val Leu Glu Arg Lys Pro Ala Ala Gly Leu Ser
50 55 60

Ala Ala Pro Val Pro Thr Ala Pro Ala Ala Gly Ala Pro Leu Met Asp
65 70 75 80

Phe Gly Asn Asp Phe Val Pro Pro Ala Pro Arg Gly Pro Leu Pro Ala
85 90 95

Ala Pro Pro Val Ala Pro Glu Arg Gln Pro Ser Trp Asp Pro Ser Pro
100 105 110

Val Ser Ser Thr Val Pro Ala Pro Ser Pro Leu Ser Ala Ala Ala Val
115 120 125

Ser Pro Ser Lys Leu Pro Glu Asp Asp Glu Pro Pro Ala Arg Pro Pro
130 135 140

Pro Pro Pro Pro Ala Ser Val Ser Pro Gln Ala Glu Pro Val Trp Thr
145 150 155 160

Pro Pro Ala Pro Ala Pro Ala Ala Pro Pro Ser Thr Pro Ala Ala Pro
165 170 175

Lys Arg Arg Gly Ser Ser Gly Ser Val Val Val Asp Leu Leu Tyr Trp
180 185 190

Arg Asp Ile Lys Lys Thr Gly Val Val Phe Gly Ala Ser Leu Phe Leu
195 200 205

Leu Leu Ser Leu Thr Val Phe Ser Ile Val Ser Val Thr Ala Tyr Ile
210 215 220

Ala Leu Ala Leu Leu Ser Val Thr Ile Ser Phe Arg Ile Tyr Lys Gly
225 230 235 240

Val Ile Gln Ala Ile Gln Lys Ser Asp Glu Gly His Pro Phe Arg Ala
245 250 255

Tyr Leu Glu Ser Glu Val Ala Ile Ser Glu Glu Leu Val Gln Lys Tyr
260 265 270

Ser Asn Ser Ala Leu Gly His Val Asn Cys Thr Ile Lys Glu Leu Arg
275 280 285

Arg Leu Phe Leu Val Asp Asp Leu Val Asp Ser Leu Lys Phe Ala Val
290 295 300

Leu Met Trp Val Phe Thr Tyr Val Gly Ala Leu Phe Asn Gly Leu Thr
305 310 315 320

Leu Leu Ile Leu Ala Leu Ile Ser Leu Phe Ser Val Pro Val Ile Tyr
325 330 335

Glu Arg His Gln Ala Gln Ile Asp His Tyr Leu Gly Leu Ala Asn Lys
340 345 350

Asn Val Lys Asp Ala Met Ala Lys Ile Gln Ala Lys Ile Pro Gly Leu
355 360 365

Lys Arg Lys Ala Glu
370

<210> 7.
<211> 199
<212> PRT
<213> Homo sapiens

<400> 7.

Met Asp Gly Gln Lys Lys Asn Trp Lys Asp Lys Val Val Asp Leu Leu
1 5 10 15

Tyr Trp Arg Asp Ile Lys Lys Thr Gly Val Val Phe Gly Ala Ser Leu
20 25 30

Phe Leu Leu Leu Ser Leu Thr Val Phe Ser Ile Val Ser Val Thr Ala
35 40 45

Tyr Ile Ala Leu Ala Leu Leu Ser Val Thr Ile Ser Phe Arg Ile Tyr
50 55 60

Lys Gly Val Ile Gln Ala Ile Gln Lys Ser Asp Glu Gly His Pro Phe
65 70 75 80

Arg Ala Tyr Leu Glu Ser Glu Val Ala Ile Ser Glu Glu Leu Val Gln
85 90 95

Lys Tyr Ser Asn Ser Ala Leu Gly His Val Asn Cys Thr Ile Lys Glu
100 105 110

Leu Arg Arg Leu Phe Leu Val Asp Asp Leu Val Asp Ser Leu Lys Phe
115 120 125

Ala Val Leu Met Trp Val Phe Thr Tyr Val Gly Ala Leu Phe Asn Gly
130 135 140

Leu Thr Leu Leu Ile Leu Ala Leu Ile Ser Leu Phe Ser Val Pro Val
145 150 155 160

Ile Tyr Glu Arg His Gln Ala Gln Ile Asp His Tyr Leu Gly Leu Ala
165 170 175

Asn Lys Asn Val Lys Asp Ala Met Ala Lys Ile Gln Ala Lys Ile Pro
180 185 190

Gly Leu Lys Arg Lys Ala Glu
195

<210> 8
<211> 473
<212> PRT
<213> Homo sapiens

<400> 8

Met Lys Arg Ala Ser Ala Gly Gly Ser Arg Leu Leu Ala Trp Val Leu
1 5 10 15

Trp Leu Gln Ala Trp Gln Val Ala Ala Pro Cys Pro Gly Ala Cys Val
20 25 30

Cys Tyr Asn Glu Pro Lys Val Thr Thr Ser Cys Pro Gln Gln Gly Leu
35 40 45

Gln Ala Val Pro Val Gly Ile Pro Ala Ala Ser Gln Arg Ile Phe Leu
50 55 60

His Gly Asn Arg Ile Ser His Val Pro Ala Ala Ser Phe Arg Ala Cys
65 70 75 80

Arg Asn Leu Thr Ile Leu Trp Leu His Ser Asn Val Leu Ala Arg Ile
85 90 95

Asp Ala Ala Ala Phe Thr Gly Leu Ala Leu Leu Glu Gln Leu Asp Leu
100 105 110

Ser Asp Asn Ala Gln Leu Arg Ser Val Asp Pro Ala Thr Phe His Gly
115 120 125

Leu Gly Arg Leu His Thr Leu His Leu Asp Arg Cys Gly Leu Gln Glu
130 135 140

Leu Gly Pro Gly Leu Phe Arg Gly Leu Ala Ala Leu Gln Tyr Leu Tyr
145 150 155 160

Leu Gln Asp Asn Ala Leu Gln Ala Leu Pro Asp Asp Thr Phe Arg Asp
165 170 175

Leu Gly Asn Leu Thr His Leu Phe Leu His Gly Asn Arg Ile Ser Ser
180 185 190

Val Pro Glu Arg Ala Phe Arg Gly Leu His Ser Leu Asp Arg Leu Leu
195 200 205

Leu His Gln Asn Arg Val Ala His Val His Pro His Ala Phe Arg Asp
210 215 220

Leu Gly Arg Leu Met Thr Leu Tyr Leu Phe Ala Asn Asn Leu Ser Ala
225 230 235 240

Leu Pro Thr Glu Ala Leu Ala Pro Leu Arg Ala Leu Gln Tyr Leu Arg
245 250 255

Leu Asn Asp Asn Pro Trp Val Cys Asp Cys Arg Ala Arg Pro Leu Trp
260 265 270

Ala Trp Leu Gln Lys Phe Arg Gly Ser Ser Ser Glu Val Pro Cys Ser
275 280 285

Leu Pro Gln Arg Leu Ala Gly Arg Asp Leu Lys Arg Leu Ala Ala Asn
290 295 300

Asp Leu Gln Gly Cys Ala Val Ala Thr Gly Pro Tyr His Pro Ile Trp
305 310 315 320

Thr Gly Arg Ala Thr Asp Glu Glu Pro Leu Gly Leu Pro Lys Cys Cys
325 330 335

Gln Pro Asp Ala Ala Asp Lys Ala Ser Val Leu Glu Pro Gly Arg Pro
340 345 350

Ala Ser Ala Gly Asn Ala Leu Lys Gly Arg Val Pro Pro Gly Asp Ser
355 360 365

Pro Pro Gly Asn Gly Ser Gly Pro Arg His Ile Asn Asp Ser Pro Phe
370 375 380

Ser Asp Asn Ala Gln Leu His Val Val Asp Pro Thr Thr Phe His Gly
115 120 125

Leu Gly His Leu His Thr Leu His Leu Asp Arg Cys Gly Leu Arg Glu
130 135 140

Leu Gly Pro Gly Leu Phe Arg Gly Leu Ala Ala Leu Gln Tyr Leu Tyr
145 150 155 160

Leu Gln Asp Asn Asn Leu Gln Ala Leu Pro Asp Asn Thr Phe Arg Asp
165 170 175

Leu Gly Asn Leu Thr His Leu Phe Leu His Gly Asn Arg Ile Pro Ser
180 185 190

Val Pro Glu His Ala Phe Arg Gly Leu His Ser Leu Asp Arg Leu Leu
195 200 205

Leu His Gln Asn His Val Ala Arg Val His Pro His Ala Phe Arg Asp
210 215 220

Leu Gly Arg Leu Met Thr Leu Tyr Leu Phe Ala Asn Asn Leu Ser Met
225 230 235 240

Leu Pro Ala Glu Val Leu Met Pro Leu Arg Ser Leu Gln Tyr Leu Arg
245 250 255

Leu Asn Asp Asn Pro Trp Val Cys Asp Cys Arg Ala Arg Pro Leu Trp
260 265 270

Ala Trp Leu Gln Lys Phe Arg Gly Ser Ser Ser Glu Val Pro Cys Asn
275 280 285

Leu Pro Gln Arg Leu Ala Asp Arg Asp Leu Lys Arg Leu Ala Ala Ser
290 295 300

Asp Leu Glu Gly Cys Ala Val Ala Ser Gly Pro Phe Arg Pro Ile Gln
305 310 315 320

Thr Ser Gln Leu Thr Asp Glu Glu Leu Leu Ser Leu Pro Lys Cys Cys
325 330 335

Gln Pro Asp Ala Ala Asp Lys Ala Ser Val Leu Glu Pro Gly Arg Pro

Gly Thr Leu Pro Gly Ser Ala Glu Pro Pro Leu Thr Ala Val Arg Pro
385 390 395 400

Glu Gly Ser Glu Pro Pro Gly Phe Pro Thr Ser Gly Pro Arg Arg Arg
405 410 415

Pro Gly Cys Ser Arg Lys Asn Arg Thr Arg Ser His Cys Arg Leu Gly
420 425 430

Gln Ala Gly Ser Gly Gly Gly Gly Thr Gly Asp Ser Glu Gly Ser Gly
435 440 445

Ala Leu Pro Ser Leu Thr Cys Ser Leu Thr Pro Leu Gly Leu Ala Leu
450 455 460

Val Leu Trp Thr Val Leu Gly Pro Cys
465 470

<210> 9
<211> 473
<212> PRT
<213> Mus musculus

<400> 9

Met Lys Arg Ala Ser Ser Gly Gly Ser Arg Leu Leu Ala Trp Val Leu
1 5 10 15

Trp Leu Gln Ala Trp Arg Val Ala Thr Pro Cys Pro Gly Ala Cys Val
20 25 30

Cys Tyr Asn Glu Pro Lys Val Thr Thr Ser Cys Pro Gln Gln Gly Leu
35 40 45

Gln Ala Val Pro Thr Gly Ile Pro Ala Ser Ser Gln Arg Ile Phe Leu
50 55 60

His Gly Asn Arg Ile Ser His Val Pro Ala Ala Ser Phe Gln Ser Cys
65 70 75 80

Arg Asn Leu Thr Ile Leu Trp Leu His Ser Asn Ala Leu Ala Arg Ile
85 90 95

Asp Ala Ala Ala Phe Thr Gly Leu Thr Leu Leu Glu Gln Leu Asp Leu
100 105 110

340 345 350

Ala Ser Ala Gly Asn Ala Leu Lys Gly Arg Val Pro Pro Gly Asp Thr
355 360 365

Pro Pro Gly Asn Gly Ser Gly Pro Arg His Ile Asn Asp Ser Pro Phe
370 375 380

Gly Thr Leu Pro Ser Ser Ala Glu Pro Pro Leu Thr Ala Leu Arg Pro
385 390 395 400

Gly Gly Ser Glu Pro Pro Gly Leu Pro Thr Thr Gly Pro Arg Arg Arg
405 410 415

Pro Gly Cys Ser Arg Lys Asn Arg Thr Arg Ser His Cys Arg Leu Gly
420 425 430

Gln Ala Gly Ser Gly Ala Ser Gly Thr Gly Asp Ala Glu Gly Ser Gly
435 440 445

Ala Leu Pro Ala Leu Ala Cys Ser Leu Ala Pro Leu Gly Leu Ala Leu
450 455 460

Val Leu Trp Thr Val Leu Gly Pro Cys
465 470

<210> 10
<211> 15
<212> PRT
<213> Artificial Sequence

<220>
<223> synthetic

<400> 10

Ser Gly Val Pro Ser Asn Leu Pro Gln Arg Leu Ala Gly Arg Asp
1 5 10 15

<210> 11
<211> 15
<212> PRT
<213> Artificial Sequence

<220>
<223> synthetic

<400> 11

Thr Arg Ser His Cys Arg Leu Gly Gln Ala Gly Ser Gly Ser Ser
1 5 10 15